PROGRESS IN THE CHEMISTRY OF FATS AND OTHER LIPIDS

Volume 2

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CONTENTS

	Page
PREFACE	vii
THE POLYMORPHISM OF GLYCERIDES	1
T. Malkin, Department of Organic Chemistry, The University, Bristol.	
AUTOXIDATION OF FATS AND RELATED SUBSTANCES	51
Ralph T. Holman, The Hormel Institute, University of Minnesota, Austin, Minnesota, U.S.A.	
NUTRITIONAL SIGNIFICANCE OF THE FATS	99
Harry J. Deuel, Jr., University of Southern California, Los Angeles, California, U.S.A.	
THE SURFACE PROPERTIES OF FATTY ACIDS AND ALLIED SUBSTANCES	193
D. G. Dervichian, Institut Pasteur, Paris, France.	
UREA INCLUSION COMPOUNDS OF FATTY ACIDS	243
H. Schlenk, Texas A. & M. College, College Station, Texas, U.S.A.	
INFRARED ABSORPTION SPECTROSCOPY IN FATS AND OILS	268
D. H. Wheeler, General Mills Inc., Minneapolis, Minnesota, U.S.A.	
COUNTER-CURRENT FRACTIONATION OF LIPIDS	292
H. J. Dutton, Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.	
NAME INDEX	327
SUBJECT INDEX	338

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NAME INDEX

ABELIN, I. 139, 186
ABRAHAM, E. P. 323
ABU-NASR, A. M. 257, 259, 266
ADAM, N. K. 206, 218, 219, 220, 224, 226, 227, 228, 229, 231, 236, 239, 240, 241
ADAMS, K. 281, 290
ADDISON, C. C. 210, 212, 239
AHMAD, K. 275, 290
AHRENS, E. H., JR. 300, 314, 315, 323, 324
ALEXANDER, A. E. 214, 224, 233, 240, 241
ALFIN-SLATER, R. B. 178, 192
ALLEN, R. R. 67, 68, 74, 96
ALYEA, H. N. 80, 96
AMES, S. R. 176, 191
ANDERSON, B. 79, 96
ANDERSON, R. J. 117, 121, 184
ANDERSON, W. E. 181
ANDERSON, W. H. 191
ANISFELD, L. 182
ANNEGERS, J. H. 160, 189
ARCHIBALD, R. C. 301, 323
ARMFIELD, F. A. 94
ARNOLD, A. 184
ARNSCHINK, L. 152, 188
ASKEW, F. A. 214, 240
ATCHLEY, W. A. 306, 324
AUGUR, V. 157, 165, 188
AULT, W. C. 95, 182, 265
AUXIER, R. W. 281, 290
AXELROD, A. E. 133, 134, 135, 185, 186
BÄCKSTRÖM, H. L. J. 80, 96
BACON, E. K. 131, 155, 172, 178, 186, 188, 191
BAILEY, A. E. 12, 49, 182, 190, 324
BAKER, R. 192
BALDWIN, A. R. 41, 49
BANERJI, G. G. 125, 184
BARKER, J. T. 234, 241
BARKI, V. H. 105, 173, 182, 190, 191
BARNARD, D. 90, 97
BARNES, R. B. 289
BARNES, R. H. 92, 98, 156, 164, 187, 189
BARNETT, C. E. 280, 290
BARRY, G. T. 324
BARTELL, F. E. 203, 212, 237, 239, 241
BARTELS, C. R. 323
BARTH, F. 189
BÄRWIND, H. 29, 49
BATEMAN, L. 61, 66, 86, 96
BAUER, S. T. 49
BAUER, W. H. 289, 291
BAUERNFEIND, J. C. 185
BAUMANN, C. A. 143, 185, 187
BAUR, F. J. 35, 37, 49, 191
BAVETTA, L. 164, 189, 191
BAXTER, J. G. 190
BEACH, E. F. 144, 187
BEADLE, B. W. 97, 281, 290
BECK, L. W. 189, 191
BECKER, G. H. 161, 189
BEHRING, H. v. 191
BENEDICT, J. H. 46, 50, 191
BENGEN, F. 243, 265
BERG, J. 139, 187
BERG, R. L. VON 323
BERGMANN, M. 29, 49
BERGQUIST, L. 187
BERGSTRÖM, S. 61, 62, 84, 95, 97, 318, 325
BERNAL, J. D. 49
BERNHART, F. W. 186
BERNHEIM, F. 87, 94, 97, 98
BERNHEIM, M. L. C. 97
BERNSTEIN, C., JR. 183
BERRY, W. A. 228, 240
BERRYHILL, F. M. 192
BERSTEIN, J. M. 324
BERTHELOT, M. 1, 48
BERTRAM, S. H. 191
BERZELIUS, J. J. 51, 94
BESMAN, L. 189
BEST, C. H. 187
BEVERIDGE, J. M. R. 179, 192
BEZMAN, I. I. 84, 97, 281, 284, 290
BIERNBAUM, A. 227, 240
BILLS, C. E. 169, 190
BINGEMANN, M. E. 187
BIRCH, T. W. 127, 181, 185
BLATHERWICK, N. R. 187
BLACK, A. 113, 183, 184
BLEWETT, M. 137, 186
BLOCH, K. 192
BLODGETT, K. 230, 240
BLOMSTRAND, E. 84, 97

Bloomfield, G. F. 95
Blum, W. P. 279, 280, 290
Blunden, H. 187
Boas, M. A. 129, 185
Boer, J. 171, 172, 173, 188, 190, 191
Bolland, J. L. 60, 61, 63, 66, 73, 75, 76, 78, 80, 95, 96, 325
Bollman, J. L. 189
Bolomey, R. A. 190
Boord, C. E. 286, 291
Booth, R. G. 132, 186
Boruff, C. S. 185
Bosworth, A. W. 155, 189
Boughton, B. W. 136, 186
Bouhet, C. 201, 234, 239
Boutwell, R. K. 172, 173, 186, 190
Bowditch, H. I. 189
Boxendale, J. H. 96
Boyd, G. E. 218, 220, 225, 240
Boyd, H. M. 96, 276, 325
Boyd, O. F. 156, 189
Boyer, P. D. 97
Bradley, T. F. 266
Bradshaw, P. J. 187
Brandt, K. 180, 192
Bratzler, J. W. 184
Brauer, R. W. 66, 70, 96
Braun, W. Q. 176, 191
Bpekke, O. L. 324
Brice, B. A. 106, 182, 260, 265
Bricker, C. E. 95, 321, 325
Briegleb, G. 249, 265
Broquist, H. P. 133, 186
Brown, C. F. 190, 191
Brown, F. E. 239
Brown, J. B. 70, 74, 95, 96, 182, 275, 290
Brown, L. E. 95
Brown, W. R. 110, 183
Browning, D. D. 284, 290
Brues, A. M. 143, 187
Brunner, H. 284, 290
Brush, M. 183
Buckley, T. A. 168, 190
Bumpus, F. M. 275, 290
Burket, S. C. 325
Burns, M. J. 131, 185
Burr, G. O. 67, 73, 92, 95, 96, 98, 99, 100, 101, 104, 105, 106, 156, 181, 182, 183, 187, 189, 190
Burr, M. M. 99, 100, 101, 105, 181, 182
Butts, J. S. 186, 187
Cahill, W. M. 184
Calbert, C. E. 181, 182, 188, 192
Callen, J. E. 274, 289
Campbell, H. L. 176, 191
Canals, E. 198, 239
Cannon, J. A. 324, 325
Carhart, H. 265
Carlson, A. J. 174, 191
Carpenter, L. E. 185
Carter, M. G. R. 2, 41, 46, 48, 49
Cary, A. 215, 240
Cason, K. 286, 290
Catravas, G. N. 263, 265
Cavier, R. 212, 235, 239, 241
Celmer, W. D. 287, 291
Cerecedo, L. R. 183
Chaikoff, I. L. 144, 188
Chaminade, R. 216, 240
Chapman, R. A. 90, 97
Charbonnet, G. H. 24, 49
Chen, C. 14, 22, 27, 49
Cheng, A. L. S. 132, 135, 136, 141, 149, 152, 155, 184, 186, 187, 188, 192
Chipault, J. R. 70, 72, 74, 77, 89, 96, 190
Chittenden, R. H. 189
Chu, T. K. 98
Church, C. F. 126, 184
Clark, G. L. 235, 239, 241
Clark, H. 183
Clarke, T. H. 34, 41, 42, 44, 45, 49, 50
Clarkson, C. E. 2, 10, 11, 16, 20, 48, 49
Clausen, M. 98
Claxton, E. 66, 96
Clayton, C. C. 143, 187
Clegg, R. E. 265
Cockbain, E. G. 232, 241
Codie, J. F. 122, 184
Coffey, R. J. 189
Cohen, S. 133, 186
Cole, P. G. 313, 324
Coleman, J. E. 95, 262, 265, 266, 325
Collet, P. 230, 241
Collins, R. A. 182
Colpa, J. P. 75, 96
Common, R. H. 192
Copeland, L. E. 225, 240
Cornbleet, T. 183
Cornish, R. E. 301, 323
Corrin, H. L. 238, 242
Coumoulos, G. D. 230, 241
Courtney, A. M. 188

Cowan, J. C. 325, 324
Cox, R. P. 89, 95
Craig, D. 323
Craig, L. C. 292, 296, 298, 300, 303, 314, 315, 323, 324
Cramer, F. 205, 266
Crampton, E. W. 178, 192
Crawford, D. 192
Crawford, M. F. 274, 289
Crockett, M. E. 191
Crombie, L. 287, 291
Cross, C. K. 275, 290
Cross, D. M. 188
Crowe, R. W. 34, 49
Cruickshank, E. M. 183
Crum, C. L. 189
Cullimore, O. S. 187
Culver, P. J. 189
Cummings, M. J. 190
Curbera, A. 264
Curtis, H. C. 185
Cutler, C. H. 187
Czaczkes, J. W. 126, 185
Dal Nogare, S. 95, 321, 325
Dam, H. 97
Danielli, J. F. 214, 240
Daubert, B. F. 1, 14, 22, 27, 32, 34, 36, 38, 41, 42, 44, 45, 46, 49, 50, 84, 97, 180, 274, 275, 281, 289, 290, 324
Davies, E. C. H. 239
Davies, L. S. 305, 324
Davis, J. E. 179, 192
Davis, J. K. 212, 239
Deatherage, F. E. 54, 55, 70, 74, 95, 96, 275, 290
Decker, A. B. 109, 141, 183, 187
Derfer, J. M. 283, 291
Derse, P. H. 190
Dervichian, D. G. 200, 213, 216, 217, 218, 220, 223, 224, 225, 228, 229, 230, 231, 232, 234, 239, 240, 241
Desnuelle, P. 202, 213, 228, 231, 239
Deuel, H. J., Jr. 101, 104, 114, 119, 132, 140, 144, 146, 149, 151, 153, 164, 171, 172, 173, 174, 175, 179, 181, 182, 183, 184, 185, 186, 187, 188, 190, 191, 192
Devaux, H. 206, 239
van Dijck, W. J. D. 302, 323
Dimler, R. J. 324
Dinerstein, R. A. 267
Dockstader, W. B. 89, 97
Doerschuk, A. P. 324
Dollear, F. G. 86, 95
Donahue, D. J. 203, 239
Donaldson, Jeanette 324
Donnan, F. G. 207, 209, 210, 234, 239, 241
Dorst, S. 122, 184
Drake, N. L. 265
Drucker, K. 233, 241
Drummey, G. D. 189
Drury, D. R. 187
Duclaux, E. 233, 234, 241
Duffy, P. 1, 2, 48
Dugan, L. R. 281, 290
Dunlop, A. K. 266
Dunn, H. J. 155, 160, 178, 188
Durbin, G. T. 186
Dutton, H. J. 323, 324, 325
Dyer, J. W. W. 226, 240
Earle, F. R. 324
Eda, K. 232, 241
Eddy, C. R. 95, 276, 290, 325
Edeleanu, G. M. B. H. 324
Edwards, W. G. H. 305, 324
Eisenberg, G. 174, 191
Ekwall, P. 229, 237, 240, 241
El Ridi, M. S. 189
Elledge, H. G. 210, 239
Elliott, R. F. 184
Ellis, G. W. 54, 95
Ellis, N. R. 109, 183
Elmer, O. 72, 74, 95
Elvehjem, C. A. 123, 129, 132, 171, 173, 182, 184, 185, 186, 190, 191
Embree, N. D. 191
Endicott, E. N. 188
Engel, R. W. 111, 183
Entenman, C. 188
Ericson, A. T. 265
Ershoff, B. H. 124, 140, 141, 174, 184, 187, 191
Euler, B. v. 171, 172, 173, 175, 190, 191
Euler, H. v. 171, 172, 175, 190, 191
Evans, H. M. 99, 100, 101, 124, 125, 126, 181, 182, 184, 190, 302, 323
Evans, M. G. 96
Ewing, M. E. 187
Faber, H. K. 183
Fahnestock, M. K. 187
Fales, H. L. 188
Farmer, E. H. 54, 86, 95, 96
Farmer, F. A. 192
Farrow, H. L. 189
Fash, R. H. 324

Ferguson, C. C. 113, 183
Ferguson, R. H. 40, 41, 49
Ferreira-Denard, L. 230, 241
Fetterly, L. C. 254, 265, 266
Feuge, R. O. 208, 239, 275, 290
Fieger, E. A. 133, 134, 136, 186
Field, E. T. 273, 275, 289, 290
Field, H., Jr. 126, 184
Fields, M. 183
Fiestas, J. 266
Filer, L. J., Jr. 14, 27, 34, 41, 49, 95
Fillerup, D. L. 183, 187
Finnerud, C. W. 183
Fischer, E. 29, 49
Fisher, E. K. 210, 239
Fisher, H. R. 192
Fitch, J. B. 183
Flitter, D. 249, 266
Flous, M. E. 239
Forbes, E. B. 115, 116, 117, 118, 184
Fountaine, F. C. 183
Foy, J. R. 183
Fraenkel, G. 137, 186
Franke, W. 54, 95, 97
Fraps, G. S. 131, 185
Frazer, A. C. 177, 189, 191
Freeman, N. K. 285, 286, 290
Freeman, S. 179, 192
Freeman, S. E. 307, 309, 324
French, C. E. 113, 116, 183, 184
Fretzdorff, A. M. 226, 240
Freudenberg, K. 265, 266
Friedgood, C. E. 113, 183
Fritz, J. C. 98
Frumkin, A. 229, 234, 240, 241
Fu, Y. 237, 241
Fugger, J. 324, 325
Fukui, T. 181, 191
Gable, C. M. 266
Galbraith, H. 188
Gamble, D. L. 280, 290
Gans, D. M. 237, 241
Gardner, J. 183, 188
Garner, W. E. 24, 49
Gavin, G. 185
Gebhart, A. I. 95
Gee, G. 61, 86, 96
Geiger, E. 113, 183
George, P. 80, 96
Gerecht, J. F. 95
Geyer, B. R. 190
Geyer, R. P. 132, 171, 173, 186, 190, 191
Giacomello, G. 266
Giblin, L. A. 189
Gibson, G. 183
Gibson, G. P. 96
Gil, G. 257, 266
Gilbert, E. C. 201, 202, 239
Gillam, A. E. 189
Gillum, H. L. 187
Gilmore, R. C. 112, 123, 124, 183
Ginsberg, J. E. 183
Givens, M. H. 155, 189
Glavind, J. 90, 97
Glickman, N. 187
Gloyer, S. W. 322, 325
Go, Y. 266
Golumbic, C. 82, 96, 97
Gooding, C. M. 190, 191
Goodman, J. G. 125, 184
Gordon, H. H. 152, 188
Gore, R. C. 289
Gortner, R. A. 237, 241
Gottleib, H. L. 190
Goursac, F. de 94
Granados, H. 97
Grant, G. A. 97
Greeley, P. O. 189
Greenberg, S. M. 93, 98, 104, 105, 106, 107, 140, 141, 145, 181, 182, 185, 186, 187, 188, 190, 191, 192
Greisheimer, E. 187
Gröer, F. von 109, 183
Groot, E. H. 75, 96, 191
Gros, A. T. 208, 239
Gross, J. B. 179, 192
Grossfeld, J. 191
Grunewald, C. F. 187
Gruntzig, W. 1, 2, 48, 49
Guastalla, J. 230, 240
Guerra, E. Z. 140, 187
Guggenheim, K. 126, 185
Guillemonat, A. 266
Guirard, B. M. 185
Gulick, M. 144, 186, 187
Gullickson, T. W. 109, 183
Gunsalus, I. C. 128, 185
Gunstone, F. D. 72, 73, 96
Gupta, A. 45, 50
Guth, F. 1, 21, 48
Guyot, J. 229, 240
Gyenge, J. M. 325
György, P. 127, 177, 181, 191
Hallman, L. F. 187, 188, 189, 191
Halpern, A. 199, 239
Halpin, J. L. 98
Halvorson, H. O. 80, 97

HAMAGUCHI, K. 226, 240
HANKINS, O. G. 183
HANSEN, A. E. 109, 183
HANSON, H. T. 98
HARDY, W. B. 206, 239
HARFENIST, E. J. 300, 323
HARGRAVE, K. R. 90, 97
HARKINS, W. D. 207, 210, 218, 220, 224, 225, 229, 232, 235, 236, 237, 238, 239, 240, 241, 242
HARPER, D. A. 182
HARPLE, W. W. 289, 291
HARRIS, P. L. 190, 191
HARRIS, R. S. 186
HARRISON, G. A. 152, 188
HARRISON, G. R. 289
HARRISON, S. A. 285, 290
HART, E. B. 171, 173, 182, 186, 190, 191
HARTMAN, L. 97
HARTMANN, S. 90, 97
HARTRIDGE, H. 204, 209, 239
HASSINEN, J. B. 136, 186
HAUGE, S. M. 185
HAUSMANN, W. 300, 323
TEN HAVE, P. 80, 96
HEETHER, M. R. 273, 274, 280, 289, 290
HEFTER, G. 95, 182
HEILBRON, I. M. 189
HEINTZ, W. 1, 21, 48
HELD, N. A. 238, 242
HELLER, S. 226, 240
HENDERSON, J. L. 75, 96, 190
HENDRICK, C. 191
HENDRICKSON, M. J. 61, 89, 95
HENICK, A. S. 91, 281, 290
HENRY, K. M. 132, 172, 186, 190
HERB, S. F. 96, 324
HERMANN, C. 243, 266
HIBSHMAN, H. J. 302, 323
HICKMAN, H. P. 184
HICKMAN, K. C. D. 190
HIGGINS, J. W. 154, 188
HILDITCH, T. P. 56, 66, 72, 73, 95, 96, 109, 167, 182, 183, 188, 190
HINSHAW, E. B. 171, 190
HIROSE, M. 211, 235, 239
HOAGLAND, R. 114, 116, 152, 154, 183, 184, 188
HÖBER, R. 189
HODSON, A. 183, 186
DE HOFFMANN, E. J. 186
HOFFMAN, F. 186
HOFFMAN, W. H. 282, 290
HOFMANN, K. 133, 189, 286, 290
HOGAN, A. G. 181, 185
HOLMAN, R. T. 62, 67, 71, 72, 74, 89, 92, 93, 95, 96, 97, 98, 105, 182, 251, 256, 257, 259, 261, 266, 324
HOLMES, A. D. 146, 148, 149, 188
HOLT, L. E. 152, 188, 189
HONN, F. J. 84, 97, 281, 290
HOOPER, J. H. 98
HOUSSAY, B. A. 177, 191
HOWARD, G. A. 136, 186
HOWE, J. R. 36, 39, 49
HOWE, P. E. 180, 192
HÜCKEL, H. 266
HUFFMAN, C. F. 183
HUGHES, A. H. 229, 231, 232, 240, 241
HUME, E. M. 104, 105, 182
HUMMEL, K. 191
HUNTEN, K. W. 193, 200, 239
HUTCHINSON, E. 207, 239
IOB, L. V. 183
IRWIN, M. H. 158, 160, 161, 189, 190
ISBELL, H. S. 183
ISEMURA, T. 226, 240
ISHERWOOD, J. J. 210, 239
ITSCHNER, K. F. 185
IVY, A. C. 160, 189
JACK, E. L. 171, 190
JACKSON, A. 67, 74, 79, 96
JACKSON, J. E. 276, 290, 325
JACKSON, F. L. 25, 30, 33, 35, 43, 49, 50, 274, 289
JAEGER, F. M. 196, 197, 198, 239
JÄGERROOS, B. H. 189
JAKOB, C. W. 237, 241
JAMES, W. H. 184
JANSEN, B. C. P. 172, 173, 188, 191
JANTZEN, E. 302, 323
JASPERSON, H. 89, 97
JEFFERSON, M. E. 49
JELLINEK, H. H. G. 233, 241
JENSEN, J. L. 190
JERCHEL, D. 54, 95
JESSOP, G. 206, 218, 220, 227, 231, 239, 241
JIMENES, J. F. 258, 266
JOGLEKAR, R. B. 1, 4, 48, 197, 239
JOHNSON, R. M. 188
JOLY, M. 216, 225, 226, 228, 232, 240, 241
JONES, C. M. 165, 189
JONES, D. C. 202, 239
JONES, G. 213, 240

JONES, R. 97
JONES, R. N. 277, 278, 289, 290
JUE, D. 190
KAPLAN, N. O. 185
KARR, A. E. 323
KARRER, P. 182, 190
KASS, J. P. 67, 73, 96, 182
KAUFMANN, H. P. 96, 168, 190, 239
KEENEY, M. 97
KEESER, E. 187
KEETON, R. W. 187
KELLER, D. C. 185
KELLER, H. 190
KELLY, E. 129, 185
KENASTON, C. B. 94, 98
KENTIE, A. 172, 188, 191
KESLER, R. L. 183
KHAINSKY, I. A. 238, 242
KHAN, N. A. 55, 62, 70, 71, 74, 86, 95, 96, 97, 275, 277, 289, 290, 317, 324
KING, A. M. 24, 49
KING, H. H. 207, 239
KIRBY, E. M. 282, 290, 321, 325
KIRK, C. M. 188
KIRSCH, P. 239
KLEIMAN, G. 323
KLEINSCHMIDT, A. W. 309, 324
KLINE, E. M. 185
KLINGHOFFER, K. A. 166, 189
KNAFO, G. 263, 265, 266
KNAPP, R. M. 282, 290, 321, 325
KNIGHT, H. B. 58, 59, 95, 97, 262, 264, 265, 266, 267, 275, 274, 276, 280, 289, 290, 325
KNOL, H. W. 191
KNOX, W. T., JR. 302, 323
KNUCHEL, M. 186
KOCH, H. P. 60, 61, 95, 96, 325
KODICEK, E. 137, 186
KOENIG, H. 182
KOHN, H. I. 87, 97
KOLP, D. G. 35, 49
KON, S. K. 132, 186, 190
KONEN, J. C. 95, 89
KRAMER, B. 189
KRANKE, E. H. 98
KRATKY, C 266
KRAYBILL, H. R. 82, 83, 97, 309, 324
KRONSTEIN, M. 281, 290
KREEGER, F. B. 49
KREHL, W. A. 177, 191
KREULA, M. 131, 185
KREWSON, C. F. 132, 186
KRINGSTAD, H. 185
KROGH, A. 121, 184
KRYDER, G. D. 187, 188
KUHRT, N. H. 279, 280, 290
KÜLZ, E. 142, 187
KUMMEROW, F. A. 67, 68, 70, 74, 79, 96, 98, 120, 184, 185
KUNKEL, H. O. 111, 183
KURKJIAN, A. 185
KÜRSTEINER, P. 186
KURTZ, FLOYD E. 324
KURTZ, G. W. 87, 97
LAING, M. E. 236, 241
LAMBERT, E. H. 187
LANCASTER, C. R. 323, 324
LANCASTER, E. B. 323
LANGMUIR, I. 209, 229, 230, 234, 239, 240, 241
LANGWORTHY, C. F. 146, 149, 188
LARSEN, C. D. 192
LARSEN, R. G. 94
LARSON, H. W. 187
LASCARAY, L. 235, 241
LASSEN, S. 155, 172, 178, 186, 188, 191
LASZT, L. 163, 164, 166, 189
LATHE, G. H. 313, 324
LAUER, W. M. 95
LAURELL, S. 84, 97
LAUTZ, H. 1, 48
LEA, C. H. 51, 90, 94, 97, 109, 183
LEASE, E. J. 185
LEASE, J. G. 185
LEATHES, J. B. 231, 241
LEICHENGER, H. 174, 191
LEK, M. 133, 239
LEMIEUX, R. U. 238, 242
LEMON, H. W. 275, 282, 290, 321, 325
LENNE, H.-U. 266
LEPKOVSKY, S. 99, 101, 124, 125, 181, 182, 184, 190
LEWIS, W. R. 91, 97
LI, T. W. 179, 192
LIE, J. 185
LIDDEL, U. 289
LIND, E. L. 238, 242
LINDEMAN, G. 171, 190
LINDHARD, J. 121, 184
LINGENFELTER, J. R. 191
LINNER, E R. 237, 241
LINSTEAD, R. P. 266
LIPMANN, F. 127, 185
LIPPINCOTT, S. W. 192
LIPS, H. J. 97
LIPTON, M. A. 184

LIVERSEDGE, N. 87, 97
LOCHTE, H. L. 303, 323
LOCKHART, E. E. 186
LONG, F. A. 236, 241
LONGENECKER, H. E. 34, 41, 49, 95, 182, 324
LOOFBOUROW, J. R. 289
LOOSLI, J. K. 175, 191
LORD, J. W. 97
LORD, R. C. 289
LORENZ, F. W. 144, 188
LOSKIT, K. 1, 21, 48
LOTTERMOSER, A. 235, 236, 237, 241
LOVERN, J. A. 313, 324
LUCAS, C. C. 187
LUCAS, R. A. 286, 290
LUND, A. 79, 96
LUNDBAEK, K. 124, 143, 184, 187
LUNDBERG, W. O. 62, 64, 74, 77, 80, 81, 86, 95, 96, 97, 182, 190, 277, 289, 290, 316, 317, 324
LUSK, G. 111, 117, 121, 183, 184
LUTTON, E. S. 1, 11, 13, 14, 20, 21, 22, 23, 24, 25, 30, 31, 33, 35, 40, 41, 43, 44, 46, 49, 50, 324
LYMAN, J. F. 152, 188, 189
LYNES, K. J. 186
LYONS, C. G. 229, 240
MAAS, O. 193, 200, 239
MACDONALD, D. G. H. 125, 184
MACKAY, E. M. 189
MACKENZIE, C. G. 104, 178, 182, 192
MACKENZIE, J. B. 182, 192
MACMILLAN, D. 324
MACY, I. C. 188
MAEDER, E. C. 184
MAGNANT, C. 232, 241
MALKIN, T. 1, 2, 8, 10, 11, 13, 14, 16, 20, 21, 23, 24, 29, 33, 34, 35, 36, 39, 41, 42, 44, 45, 46, 48, 49, 50
MANN, F. C. 142, 187, 189
MANNERING, G. J. 126, 184
MARKLEY, K. S. 138
MARROWITZ, J. 142, 187
MARSDEN, J. 227, 231, 240, 241
MARTIN. A. J. P. 302, 323
MARTIN, G. J. 104, 182
MARTIN, J. B. 191
MARTÍNEZ, C. 177, 191
MASELLI, J. A. 258, 266
MASKELINE, N. S. 1, 48
MATTEI, G. 232, 241
MATTIL, K. F. 154, 188, 195
MATTILL, H. A. 190
MATTILL, H. A. 54, 95, 96, 190
MATTSON, F. H. 176, 177, 187, 190, 191
MAX, R. A. 55, 95, 275, 290
MAYNARD, L. A. 174, 183, 191
MAZUELOS, F. 266
MCAMIS, A. J. 100, 181
MCATEER, J. H. 302, 323
MCCAY, C. M. 149, 152, 153, 188
MCCLENAHAN, W. S. 266
MCCOLLUM, E. V. 182, 192
MCCUTCHEON, J. W. 274, 289
MCDOUGALL, E. J. 166, 189
MCFARLANE, W. D. 90, 97
MCGAVACK, T. H. 113, 183
MCGUIRE, T. A. 324
MCHENRY, E. W. 125, 184, 185
MCKAY, A. F. 277, 278, 289, 290
MCKERCHER, B. H. 190
MCLAUGHLIN, R. L. 266
MCLEAN, J. R. 179, 192
MCMASTER, T. D. 187
MCNAMARA, H. 152, 188
MCQUARRIE, I. 183
MEAD, J. F. 86, 97, 183, 187
MEADE, E. M. 247, 254, 258, 261, 264, 265, 266
MEARA, M. L. 2, 23, 24, 35, 44, 48, 49, 190
MEDES, G. 123, 185
MEHL, J. W. 186
MEIGS, E. B. 191
MEINKE, W. W. 131, 185
MELLON, M. G. 289
MELNICK, D. 125, 182, 184, 185, 186
MELVILLE, D. B. 185
MELVIN, E. H. 324
MENDEL, L. B. 181
MERIGOUX, R. 202, 228, 239
MEYER, J. 189
MICHAELIS, L. 81, 97
VON MIKUSCH, J. D. 277, 290
MILLAR, J. 178, 192
MILLAR, R. W. 266
MILLER, A. B. 66, 96
MILLER, E. S. 101, 105, 182, 187, 189
MILLER, H. G. 190
MILLER, J. G. F. 229, 240
MILLER, W. B. 190
MITCHELL, H. H. 132, 142, 172, 173, 186, 187, 191
MITCHELL, J. H., JR. 82, 83, 97
MITCHELL, J. S. 229, 240

Mitz, M. 186
Molines, J. 202, 213, 228, 231, 239
Morehouse, M. G. 149, 182, 184, 188
Moreno, J. M. 258, 266
Morrell, R. S. 66, 96
Morris, H. P. 192
Morrisson, J. L. 238, 242
Movitt, E. 187, 190, 191
Mozingo, R. 265
Muelder, K. D. 129, 185
Mueller, A. C. 266
Mueller, J. H. 186
Muñoz Ortiz, E. 187
Murlin, J. R. 117, 184
Murphy, E. A. 181, 184, 302, 323
Murray, S. 187, 188, 189
Myers, G. S. 278, 289, 290
Myers, J. E. 67, 68, 70, 73, 96
Myers, R. J. 229, 240
Nabori, H. 94
Nagli 84, 97
Nasini, A. 232, 241
Nasset, E. S. 280, 290
Nath, H. 173, 190, 191
Neale, S. 188, 189
Necheles, H. 189
Newey, H. A. 266
Newton, G. G. F. 323
Ney, W. O. 303, 323
Nicholls, R. V. V. 282, 290
Nichols P. J. 296, 323
Nichols, P. L. 96, 324
Nicholson, J. T. L. 134
Nickell, C. 74, 77, 96, 317, 324
Nieft, M. L. 132, 186
Nietz, A. H. 206, 239
Nightingale, G. 131, 186
Noble, W. R., Jr. 266
Noguchi, M. 94
Nord, F. F. 258, 266
Norris, F. W. 186
Norris, T. A. 324
Novelli, G. D. 185
Nowakowski, A. 201, 206, 239
Nunn, J. R. 287, 291
Nunn, L. C. A. 105, 111, 182, 183
Nutting, G. C. 236, 241
O'Connor, R. T. 86, 95, 273, 275, 289, 290
O'Daniel, L. 87, 97
O'Grady, M. P. 191
Öhnell, R. 189
Okey, R. 187
Oleson, J. J. 186
Oppel, T. W. 133, 186
Orr, W. J. C. 61, 63, 95
Orsini, D. 184
O'Shea-Elsom, K. 184
Othmer, P. 1, 48
Pace, E. R. 183
Pan, H. P. 184
Panzer, F. 113, 116, 183, 184
Paquot, M. C. 94
Parker, W. E. 259, 261, 266
Parsons, L. B. 87, 97
Paschke, R. F. 76, 77, 96, 276, 277, 290, 325
Passino, H. J. 325
Patterson, J. M. 187
Patton, S. 87, 97
Paul, H. 149, 152, 153, 188
Pauling, L. 264, 266
Pavcek, P. L. 98, 129, 185
Pearson, P. B. 113, 116, 183, 184
Pedelty, W. H. 109, 183
Pepper, M. B., Jr. 275, 290
Perry, E. S. 279, 280, 290
Peters, V. 21, 49
Peters, R. A. 204, 209, 239, 242
Petrov, V. 21, 49
Phelps, H. J. 238, 242
Phillips, E. O. 96
Pickett, E. E. 286, 291
Pillet, J. 231, 241
Platz, B. R. 185
Pockels, Agnes, 214
Podbielniak, W. J. 323, 325
Pollock, M. R. 136, 186, 238
Polskin, L. J. 183, 185
Ponsford, A. P. 187
Pool, M. F. 97
Port, W. S. 258, 267
Post, O. 298, 323
Potter, R. L. 127, 185
Potts, H. G. 210, 239
Potts, W. M. 257, 259, 266
Pound, J. R. 239
Powney, J. 210, 212, 236, 239
Prater, A. N. 97
Prill, E. A. 87, 97
Primrose, M. F. 156, 189
Privett, O. S. 63, 74, 77, 82, 90, 96, 97, 277, 289, 290, 316, 317, 324
Quackenbush, F. W. 82, 91, 92, 96, 97, 98, 120, 175, 184, 185, 190
Quimby, O. T. 21, 22, 49
Raison, M. 236, 241
Ranaivo, R. 239

Randall, H. M. 289
Randolph, P. 98
Rao, P. C. 274, 275, 289
Rasmussen, E. 174, 191
Ravich, G. 21, 41, 49
Rebello, D. 275, 290
Redlich, O. 251, 266
Reid, D. F. 190
Reifman, A. 186
Reimer, C. L. 1, 21, 48
Reinbold, Catherine L. 324
Reinecke, R. M. 112, 123, 183, 184
Reinhold, J. G. 184
Reiser, R. 105, 109, 182
Reitstötter, J. 194, 239
Rewadikar, R. S. 29, 49
Ribas, I. 257, 264, 266
Ricciuti, C. 97
Richardson, C. H. 323
Richardson, L. R. 128, 181, 185
Rideal, E. K. 200, 215, 225, 227, 229, 230, 231, 232, 233, 234, 239, 240, 241
Ridout, J. H. 187
Rieckehoff, I. G. 105, 182
Riemenschneider, R. W. 41, 49, 94, 96, 324
Ripley, W. E. 190
Rittenberg, D. 126, 182, 184, 192
Roberts, D. B. 183
Roberts, L. E. 239
Roberts, M. H. 233, 241
Roberts, S. 124, 184
Robertson, A. 96
Robeson, C. D. 190
Rockwood, E. W. 188
Rodriguez, R. R. 177, 191
Roffo, A. H. 192
Rogers, C. S. 113, 143, 183
Rojinsky, A. 238, 242
Roland, H. 193, 239
Rollman, H. S. 188
Roncero, A. V. 258, 266
Rönnestam-Säberg, I. 175, 190
Rose, C. S. 177, 191
Rosenberg, H. R. 190
Rosenberg, S. 227, 240
Ross, J. 55, 95, 237, 241
Rowntree, J. I. 185
Roy, A. 155, 178, 179, 189
Rubin, S. H. 134, 186
Rubner, M. 184
Rudolff, E. von 258, 266
Rusoff, I. I. 98, 189
Russell, W. C. 109, 129, 131, 183, 185
Ruthven, C. R. 313, 324
Ruys, A. H. 302, 323
Ryan, A. E. 189
Ryan, M. 192
Säberg, I. 172, 190, 191
Sakurai, H. 254, 266
Salmon, W. D. 113, 125, 127, 128, 129, 183, 184, 185
Sammons, H. G. 177, 191
Samochwalov, K. N. 238, 242
Samuels, L. T. 112, 123, 124, 183, 184, 187, 189
Sando, C. E. 41, 49
Sarma, P. S. 128, 185
Sato, Y. 324
Saunders, L. 202, 239
Savage, E. E. 156, 181, 182, 185, 189, 191
Sawyer, S. D. 187
Sax, S. M. 286, 290
Scanlon, J. T. 95
Schaefer, V. J. 229, 240
Schaeffer, B. B. 182, 265
Schantz, E. J. 132, 171, 172, 186, 190
Scheer, B. T. 116, 120, 121, 122, 123, 183, 184
Scheibel, E. G. 302, 323
Scheij, L. T. 1, 48
Scheiner, J. 134, 183
Schiessler, R. W. 249, 266
Schladitz, E. 237, 241
Schlenk, H. 251, 256, 261, 266
Schlenk, W., Jr. 243, 248, 249, 252, 266
Schneider, H. A. 128, 185
Schoenheimer, R. 126, 182, 184
Schofield, R. K. 234, 241
Scholfield, C. R. 324
Schönfeld, H. 95, 182
Schönheimer, R. 191
Schotz, M. C. 192
Schulman, J. H. 225, 228, 229, 231, 232, 233, 240, 241
Schwimmer, D. 113, 183
Scott, T. A., Jr. 324
Seelich, F. 207, 239
Serralach, J. A. 213, 240
Severance, E. 156, 188
Sevigne, F. J. 129, 130, 131, 185
Shapiro, O. W. 97
Shedlowsky, L. 237, 241
Sheldon, W. P. H. 152, 188

Shen, T. 180, 192
Shepherd, M. L. 188
Sherman, H. C. 176, 185, 191
Shimoda, F. 192
Shimomura, T. 211, 235, 239
Shokal, E. C. 266
Short, J. F. 302, 303, 323
Shorter, S. A. 210, 212, 239
Shreve, O. D. 273, 274, 280, 289, 290
Shrewsbury, C. L. 176, 191
Shull, G. M. 98, 129, 185
Shurbagy, M. R. el 29, 33, 35, 49
Sidhu, S. S. 14, 27, 32, 34, 36, 38, 41, 46, 49, 50
Siminovitch, L. 238
Simmer, A. 191
Sinclair, R. G. 101, 149, 182, 188, 277, 278, 289, 290
Singleton, W. S. 24, 49, 273, 289
Sivickes, P. B. 188
Skellon, J. H. 54, 79, 95, 96, 266
Smedley-MacLean, I. 105, 111, 182, 183, 187
Smith, A. E. 248, 249, 266
Smith, F. A. 182
Smith, F. G. 78, 79, 96
Smith, H. H. 182
Smith, V. F. 184
Smyth, C. P. 34, 49
Snell, E. E. 133, 185, 186
Snider, G. G. 114, 116, 152, 154, 183, 184, 188
Snyder, J. C. 186
Sobel, A. E. 161, 189
Sobotka, H. 227, 240, 266, 285, 290
Solomons, I. A. 287, 291
Soodak, M. 127, 185
Sørenson, N. A. 71, 69
Sorge, H. 267
Soskin, S. 142, 187
Sotier, A. L. 185
Soule, D. F. 122, 185, 184
Spalloni Cialdea, W. 187
Spichtin, W. 186
Stallberg-Stenhagen, S. 240
Stamm, J. 97
Starling, E. H. 100, 181
Steadman, L. T. 66, 70, 96
Steenbock, H. 158, 184, 185, 189, 190
Stene, S. 323
Stenhagen, E. 220, 221, 224, 227, 230, 232, 240, 241
Stevenson, J. A. F. 124, 143, 184, 187
Stewart, W. C. 149, 188
Stirn, F. E. 184
Stirton, A. J. 94
Stoll, A. 315, 324
Stotz, E. 78, 79, 96, 127, 185
Straub, E. 190, 191
Strich, A. 266
Strong, F. M. 185, 275, 290
Stynler, F. E. 285, 290
Subbarow, Y. 186
Sumrell, G. 286, 290
Sundralingam, A. 95
Sure, B. 174, 191
Sutton, D. A. 54, 61, 86, 95, 96
Swain, L. A. 190
Swain, M. L. 182, 265
Swanson, P. 111, 183
Swenseid, M. E. 98
Swern, D. 54, 95, 97, 247, 252, 255, 256, 258, 259, 261, 262, 264, 265, 266, 267, 273, 274, 276, 280, 289, 290, 318, 325
Swift, C. E. 54, 55, 86, 95, 184
Swift, R. W. 113, 116, 117, 118, 183, 184
Synge, R. L. M. 302, 323
Szyszkowski, B. von 233, 241
Talesnik, J. 186
Tammann, G. 21, 49
Tange, U. 182
Tanner, F. W., Jr. 324
Tappel, A. L. 87, 97
Taub, S. J. 183
Täufel, K. 83, 97
Taylor, C. G. 266
Taylor, H. S. 81, 97
Taylor, M. W. 183, 185
Templeton, H. A. 124, 141, 184
Templin, V. M. 189
Teorell, T. 214, 228, 240
Tereleski, J. T. 182
Terry, D. E. 324
Tesch, W. 235, 236, 241
Thacker, E. J. 184
Thomas, J. W. 191
Thomas, B. 188
Thorpe, R. E. 94
Thurston, J. L. 190
Tidwell, H. C. 161, 188, 189
Tiedt, J. 267
Tolberg, W. E. 86, 96, 97, 276, 277, 285, 289, 290, 317, 324, 325
Tordai, L. 207, 239
Trager, W. 133, 134, 137, 186

Traube, J. 233, 234, 241
Treybal, R. E. 323
Trillat, J. J. 201, 206, 239
Trupp, H. Y. 191
Truter, E. V. 249, 250, 251, 264, 267
Tucker, D. R. 284, 290
Turer, J. 94
Turner, H. A. 228, 240
Turpeinen, O. 104, 105, 182
Tuttle, L. C. 185
Tuzson, P. 189
Twigg, G. H. 302, 303, 323
Ubbelohde, L. 94
Umbreit, W. W. 128, 185
Umstätter, H. 194, 239
Vars, H. M. 113, 183
Verzár, F. 163, 164, 166, 189
Virtanen, A. I. 131, 185
Vogel, A. I. 195, 199, 239
Volmer, M. 6, 49
Volnova, V. 21, 49
De Vries, T. 91, 97
Wachs, W. 194, 239
Walden, P. 197, 239
Walker, H. A. 185
Ward, A. F. H. 267, 239
Waters, W. A. 66, 95, 96
Watson, H. E. 1, 4, 29, 48, 49, 197, 239
Weber, J. 158, 189, 190
Weber, W. H. 279, 280, 290
Week, E. F. 129, 130, 131, 185
Weeks, H. J. 302, 323
Weitkamp, A. W. 267
Weitzel, G. 226, 240
Welch, E. A. 279, 280, 290
Welch, H. L. 274, 289
Weygand, C. 1, 48
Whalley, M. 266
Wheeler, D. H. 41, 49, 76, 77, 83, 90, 91, 97, 276, 277, 289, 290, 317, 325, 327
Wheland, H. 267
Whipple, D. V. 126, 184
White, E. A. 109, 183
White, J. W., Jr. 324
White, M. D. L. 97
Whitehill, A. R. 186
Wiberley, S. E. 289, 291
Wick, A. N. 189
Widmer, C. 98, 105, 182
Wiegandt, H. F. 323
Wieland, H. 267
Wiese, H. F. 183
Wilbrandt, W. 189
Wilbur, K. M. 94, 97, 98
Will, W. 1, 21, 48
Wille, R. L. 25, 49
Williams, H. H. 152, 188, 191
Williams, J. N., Jr. 111, 183
Williams, V. 289
Williams, V. R. 133, 134, 136, 186
Williams, W. L. 133, 186
Williamson, B. 323
Williamson, R. 100, 182
Willits, C. O. 91, 97
Willman, W. 183
Willstätter, R. 315, 324
Wilson, B. R. 41, 42, 44, 50
Wilson, C. E. 281, 290
Wiseblatt, L. 192
Witnauer, L. P. 264, 266, 267
Witten, P. 93, 98, 266
Wojahn, J. 226, 240
Woltemate, M. L. 275, 290
Woog, P. 206, 239
Woolley, D. W. 324
Wu, E. 187
Yokela, E. 187
Young, H. A. 75, 96
Young, J. F. 218, 220, 225, 240
Yuasa, D. 191
Yudkin, J. 125, 184
Zain, H. 140, 187
Zakon, S. J. 183
Zechmeister, L. 189, 324
Zeller, J. H. 183
Zialcita, L. P., Jr. 132, 172, 173, 186, 191
Zilch, K. T. 324, 325
Zimmerschied, W. J. 267
Zscheile, F. P. 324
Zuidema, H. H. 94
Zurinov, C. 21, 41, 49

SUBJECT INDEX

ADHESION work (erg/cm²), esters and oils 205
— — fatty acids 209
Adrenal cortex, role in fat absorption 163, 164
Aedes aegypti, fatty acids as required growth factors 137
Alkyl esters, parachor of 199
— — surface tension of 195
Alloxan, toxicity of, influence of type of dietary fat 177
Antioxidant activity, mechanism of 81
Arachidonate, dermatitis and 109
— eczema and 110
— growth requirement 105, 106
— in blood serum 110
Arachidonic acid, biopotency of 101, 102, 105
— — in fat deficiency, 102, 105
— — growth requirements of lower organisms, 101, 102, 105
Aspartic acid, growth requirement for micro-organisms 133
— — growth stimulation of *Neurospora* 137
"Associative dynamic action", chart of 119
— — — fat utilization and 116
Autoxidation, mechanisms of 52
— acetylenic compounds 70
— cetane 52
— emulsions 85
— ethyl oleate 55
— glycerides 72
— lauric acid 53
— matricaria ester 71
— methyl 9,10-di-deutero oleate 55
— methyl elaidate 54
— methyl eleostearate 66
— 9,10-methyl linoleate 67
— 10,12-methyl linoleate 67
— methyl oleate 55, 58, 59
— methyl stearolate 55
— mixtures 73
— monoethenoid compounds 53
— oleic acid 53
— β-oxidation 53
— polyethenoid compounds, conjugated 66
Autoxidation, polyethenoid compounds, non-conjugated 59
— polymerization 70
— sodium linoleate 87
— 10,11-undecylenic acid 58
Autoxidation, products of, chromatographic separation of 61, 62
— — — methyl hydroperoxido-octadecenoate 54
— — — methyl hydroperoxido oleate 54
— — — moloxide 53
— — — oxido-stearic acid 54
— spectral changes in 60, 61, 91

BIOTIN, fat metabolism and 129
— growth requirements for micro-organisms 129
— oleic acid as substitute 133, 134
Blood lipids, cholesterol content as related to sex 145
— — eczema and 109, 110
— — neutral fat content as related to sex 145
— — phospholipid content as related to sex 145
— — sex differences in fat components 145

CALCIUM salts, dietary, effect on digestibility of fats 155, 156
Caprylic acid, thiamin-sparing action 125
Carbohydrate, dietary, protein metabolism and 111, 112, 114
— — survival time and 123
— — work capacity and 123, 124
Carotene, in margarine 168
— in vegetable fats and oils 168
— utilization 131
— — effect of carrier 131
— — mineral oil 131
Chaulmoogric acid, absence of biopotency in fat deficiency 103
— — glycerides of 45
Chemical structure, relation to infrared absorption 271

Chemical structure, carbon-carbon double bond stretching, wavelength of infrared absorption band 272
— — carbon-hydrogen bonds, wavelength of infrared absorption bands 271
— — carbon-hydrogen bond of aldehydes, wavelength of infrared absorption bands 272
— — carbon-hydrogen bond of nonconjugated *trans* double bonds, wavelength of infrared absorption bands 272
— — carbon-hydrogen bond of terminal acetylenes, wavelength of infrared absorption bands 272
— — carbon-hydrogen bond of trans-trans conjugated dienes, wavelength of infrared absorption bands 272
— — carbon-nitrogen bond (of nitriles), wavelength of infrared absorption bands 273
— — carbon-oxygen bonds, wavelength of infrared absorption bands 273
— — carbonyl bonds, wavelength of infrared absorption bands 272
— — oxygen-hydrogen bonds, wavelength of infrared absorption bands 273
Cholesterol deposition, following fat ingestion 177, 178
Choline, lipotropic effect, linoleic acid and 111
— metabolism, linoleic acid and 111
Clostridium butyricum, growth requirements 133
— — unsaturated fatty acids 133
Clostridium tetani, growth requirements, oleic acid 133
Clostridium welchii, growth requirements, oleic acid 133
Coefficient of digestibility 145, 147, 148
— — — factors influencing, age 152
— — — — — — concomitant foodstuffs 155
— — — — — — melting point 145, 150
— — — — — — polymerization 154, 155
— — — — — — sex 152
— — — — — — species 145
— — — — — — structure 154
Co-enzyme A, in fatty acid synthesis 127
— — requirement in formation of acetoacetate 127
Cold stress, dietary fat as protective agent 142
Conjugated di and trienes, *cis* and *trans* isomers, wavelength of infrared absorption bands 276
— eleostearates, *cis* and *trans* isomers, wavelength of infrared absorption bands 276
Corynebacterium diphtheriae, growth requirements, oleic acid 133
Countercurrent batch extraction, multiple stage, analysis of procedure 293
— — — — — — binomial expansion for 294
— — — — — — desirable characteristics of solvents for 296
— — — — — — prediction of degree of separation in 295
Countercurrent distribution, apparatus for 298
— — definition of 292
Countercurrent distribution, application of, to bile acids 314
— — — — — to fat acids 316
— — — — — to fat acids (oxidation products) 309
— — — — — to glycerides 303
— — — — — to methyl esters of fat acids 306
— — — — — to phospholipids 311
— — — — — to pigments 315
Countercurrent fractionation, fundamentals of 292

7-Dehydrocholesterol, conversion to vitamin D by irradiation 169
Dermatitis, fat deficiency and 109, 127
— pyridoxine deficiency and 127
1,2-Diglycerides, polymorphism of 39
1,3-Diglycerides, polymorphism of 35, 45, 46, 47, 48
Dihydrositosterol, conversion to vitamin D by irradiation 169
2,4-Dinitotoluene (DNT), response to dietary fat 143
— toxicity of 143
Dioxidostearic acid, absence of biopotency in fat-deficiency 103

Docosahexaenoic acid, biopotency in fat deficiency 102
4,8,12,15,19-Docosapentaenoic acid, absence of biopotency in fat deficiency 103
Double bonds, *trans*, detection of isolated 274
— — elaidic acid, infrared and Raman spectra of ethyl esters of 274
— — linoleic acid, infrared and Raman spectra of ethyl esters of 274
— — linolelaidic acid, infrared and Raman spectra of ethyl esters of 274
— — linolenic acid, infrared and Raman spectra of ethyl esters of 274
— — oleic acid, infrared and Raman spectra of ethyl esters of 274
— — 11-octadecenoic acid, *cis* and *trans*, wavelength of infrared absorption bands 275
— — vaccenic acid, wavelength of infrared absorption bands 274

ECZEMA, as sympton of fatty acid deficiency 109, 110
α-Elaeostearic acid, absence of biopotency in fat deficiency 103
11,14-Eicosadienoic acid, absence of biopotency in fat deficiency 103
Elaidic acid, absence of biopotency in fat deficiency 102
— as growth factor for bacteria 134
Ephestia kuehniella (flour moth), growth requirements 137
— — — — — — — arachidonic acid 139
— — — — — — — linoleic acid 137
— — — — — — — α-tocopherol 137
Ergosterol, conversion to vitamin D by irradiation 169
Erucic acid, absence of biopotency in fat deficiency 102

FAT, absorption rate 157
— — — comparative absorption of various fats 158
— — — effect of previous fasting 158
— — — effect of, on digestibility 159, 160
— — — factors influencing, adrenocortical hormones 163, 164
— — — — — — age 160, 161
— — — — — — emulsifying agents 165
Fat, absorption rate, factors, influencing, indices 158
— — — — — — inhibitors 166
— — — — — — — monoiodoacetic acid 166
— — — — — — — phlorhizin 166
— — — — — — lecithin 165
— — — — — — melting point 159, 163
— — — — — — nature of the fat 161
— — — — — — — — — fatty acids 162, 163
— — — — — — — — — triglycerides 161, 162
— — — — — — nutritional value of fat 157, 158, 60
— — — — — — polyoxyethylene sorbitan monooleate 165
— — — — — — sex 161
— — — Frazer Partition Theory 161 162
— — — Verzár Lipolytic Theory 161, 162
— adsorption of, following subtotal gastrectomy, effect of PSM 165
— — — in sprue, effect of PSM 165
— animal, digestibility of 145
— — — — — coefficient of 145, 147
— — — — — — — effect of melting point 145, 146, 148
— — essential fatty acid content 108, 170
— antirachitic effect 132
— — — in relation to calcium excretion 132
— as carrier of fat-soluble vitamins 99, 129
— as source of essential fatty acids 100
— caloric density of 99
— caloric value in relation to other foodstuffs 117
— deficiency, symptoms of 100, 109, 110, 116
— — therapeutic value of purified fatty acids 102, 103
— dietary, contraindications 179
— — effect on response to stress 139
— — human requirement 180, 181
— — optimum levels 179
— — possible deleterious effects, cholesterol deposition 177
— — — — — toxicity due to heating 178, 179

Fat, dietary, possible deleterious effects, toxicity due to rancidity 179
— — protective effect following hepatectomy 142
— — — — — in cold stress 142
— — — — — in thyrotoxicosis 139, 140
— — — — — in x-radiation 141
— — protein metabolism and 111, 112, 113, 114
— digestibility 145
— — animal *vs* vegetable fats 145, 170
— — as related to nutritional value 145
— — coefficient of 145, 147
— — factors influencing, age 152
— — — — — calcium salts in diet 155, 156
— — — — — concomitant foodstuffs 155
— — — — — dietary protein 156, 157
— — — — — hydrogenation 146, 148
— — — — — melting point 145
— — — — — polymerization 154, 155
— — — — — rate of absorption 159, 160
— — — — — sex 152
— — — — — species 145
— — — — — structure 154
— — in animals other than man 149
— — in man 145
— energy requirements 116, 118, 121, 122, 123
— functions of 99, 100
— growth requirements 100, 114
— heated, toxicity of 178, 179
— hydrogenated, digestibility of 146
— — essential fatty acid content 108
— — fatty acid deficiency and 128
— — vitamin E content 170
— in synthesis of folic acid 129
— in synthesis of niacin 129
— metabolism, biotin and 129
— niacin-sparing action 129
— nutritional value, animal *vs* vegetable fats 173–4
— — — "associative dynamic action" 116
— — — comparative value of animal and vegetable fats 167
— — — eczema and 109, 110
— — — energy requirements and 116, 121
— — — factors altering 143
— — — — — absorption rate 157
— — — — — digestibility and absorption 145
Fat, nutritional value, factors altering, glyceride structure 176, 177
— — — — — — sex 143, 144
— — — — — — source 167
— — — — — — type of fat 177
— — — galactose absorption and 132
— — — glyceride structure 176, 177
— — — growth and 114, 139
— — — hepatectomy and 113
— — — lactation and 120, 139
— — — lactose metabolism and 132
— — — "multigeneration tests" of various fats 176
— — — nitrogen, urinary and 111
— — — pregnancy and 120, 139
— — — protein metabolism and 111
— — — sexual maturity and 119
— — — — — — — relation to fat intake 120, 121
— — — "specific dynamic action" 117
— — — survival time and 123, 124
— — — thiamin requirement and 124, 125
— — — thyrotoxicosis and 139
— — — vitamin requirements and 124, 125, 126, 127, 128
— — — — — — — vitamin A and provitamin A in animal and vegetable fats 167, 168
— — — — — — — vitamin D in animal and vegetable fats 168, 169
— — — — — — — vitamin E in animal and vegetable fats 169, 170
— — — work capacity and 121, 122 123
— oxidation, in relation to tocopherol content 170
— polymerization, effect on digestibility 154, 155, 178, 179
— pyridoxine-sparing activity 99, 127
— rancidity, toxicity due to 51
— requirements, human 181, 182
— — sex differences in 143
— riboflavin-sparing activity 99, 129
— utilization, sex differences in 143
— vegetable, digestibility of, 145
— — — — coefficient of, 145, 147
— — — — effect of melting point 145
— — essential fatty acid content 108, 170

Fatty acids, absorption rate 162, 163
— — adsorption from solution (air-water interface) 233
— — adsorption from solution (solid-liquid interface) 237
— — area for chain of saturated 221
— — average coefficients of digestibility in animals 151
— — biotin-like activity 138
— — — — — formula for calculation of 137
— — deficiency, relative requirements of various animals 109, 110
— — — therapeutic effect of unsaturated fatty acids 102, 103
— — essential, biopotency of 104
— — — comparative amounts in animal and vegetable fats 107, 108, 109, 170
— — — growth requirements 110
— — — — — for bacteria 133
— — — — — *cis*-octadecenoic acids 135
— — — — — *trans*-octadecenoic acids 135
— — human requirements 109
— — metabolic effects 110, 111
— — methods for determining 106
— — — — — bioassay 106
— — — — — chemical 106
— — — — — hydrogenation 107
— — neoplasm and 111
— — nutritional requirements 100, 104, 105, 109
— — parachors of 199
— — propylactic and curative effect of 101, 102, 103
— — requirements, sex differences in 145
— — saturated, bacterial growth and, synergistic action 136
— — saturated and unsaturated, wavelength of infrared absorption bands 273
— — surface tension of 193
— — synthesis of, by thiamin 126
— — from carbohydrate 126
— — — — pantothenic acid and 127
— — — — pyridoxine and 128
— — unsaturated, bacterial growth and 133, 135
— — — bacteriostatic effect 137
— — — — — reversal of, by lecithin 137
Fatty acids, unsaturated, bacteriostatic effect of, reversal of, by protein 137
— — — — — — — by sterols 137
— — — — — — — by tocopherol 137
— — — in animal vs vegetable fats 170
— — — therapeutic value in fat deficiency 101
— — urea inclusion compounds of 243
— — — — — — composition of 244, 253
— — — — — — dissociation temperatures of adducts 250
— — — — — — fractionation, by means of 257
— — — — — — heat of adduct formation 247
— — — — — — preparation and analysis of 251
— — — — — — preparation of pure unsaturated acids and esters 261
— — — — — — properties of 255
— — — — — — recovery of fatty constituents 253
— — — — — — resistance to oxidation 256
— — — — — — theory of 244
— — — — — — use in determining structures 263
— — — — — — x-ray data 249
Fatty acids and esters, interfacial tension against water of solutions 207
Fatty substances, adsorption and orientation at the surface of their solution in oils or organic solvents 201
— — adsorption at water-oil interfaces 209
— — Eötvös constant of 200
— — influence of *p*H on interfacial tension between water and 204
— — interfacial tension between water and 203
— — orientation at the contact with water 206
— — orientation at the surface of 201
— — spreading at water oil interface 213
Folic acid, fat and 129
— — synthesis of 129

GALACTOSE absorption, fat and 132
— — metabolism, role of fat 132
Gastrectomy, subtotal, fat absorption following, effect of PSM 165
Glycerides (*see also* Polymorphism) comparative nutritive value of mono-, di-, and triglycerides 176, 177
— cooling and heating curves 3
— determination of capillary melting points 8
— long X-ray spacings 10
— short X-ray spacings 13
— thermal and X-ray investigation 2
— the vitreous form 20
— X-ray investigation 9
Glycogen, sex differences in liver storage 144
Growth factors (*see also* Biotin) fat requirements 100, 114, 115
Growth hormone, factors influencing effectiveness 174
Growth of children, in relation to fat fed 174
Growth tests, with animal *vs* vegetable fats 170
— — in children 174
— — in normal rats 171
— — — — — — effect of vaccenic acid 172, 173
— — in rats on restricted diets 173, 174
— — in rats prematurely weaned 173
— — in rats receiving growth hormone 174

HAEMOGLOBINURIA, due to alloxan 177
— — — — protective effect of tocopherol 177
Heat production, various foodstuffs and 117, 118
Hepatectomy, partial, protective effect of dietary fat 143
Hexahydroxystearic acids, biopotency in fat deficiency 102
Hormones, adrenocortical, in fat absorption 163, 164
Hypothalamus, lesion of, 143
— — — hyperphagia due to 143
— — — — — — — response to dietary fat 143

INFRARED absorption spectroscopy, fats and oils 268
Infrared absorption spectroscopy, ininstruments 270
— — — molecular rotation frequencies 268
— — — units of measurement 270
Infrared absorption of aluminium soaps 289
— — autoxidation of unsaturated oils 280
— — cyclopropane ring 286
— — epoxy compounds 280
— — fatty acids, branched 285
— — herculin 287
— — hydroperoxide groups 280
— — lacto-bacillic acid 286
— — monoglyceride, fatty acid 278
— — oil-styrene polymers 284
— — phthenoic acids 286
— — sterculic acid 287

KETONURIA, endogenous 144
— — sex differences in 144
— exogenous 144
— — as index of fat metabolism 144
— in alloxan diabetes 144
— — — — — sex differences in 144

LACTATION, fat intake and 120
— — — — — animal *vs* vegetable fats 174
Lactobacillus arabinosus, growth requirements 133
— — — — aspartic acid 133
— — — — octadecenoic acids 135
— — — — unsaturated fatty acids 133, 135, 136
Lactobacillus bulgaricus, growth requirements 133
— — — — oleic acid 133
Lactobacillus fermenti, growth requirements 133
— — — — unsaturated fatty acids 133
Lactose, metabolism, role of fat 132
Lecithin, as emulsifying agent, effect on absorption of fats 165
Linoelaidic acid, absence of biopotency in fat deficiency 103
Linoleate, dermatitis and 109
— eczema and 110
— growth requirements 104, 105, 107, 128
— — — chart of 107

Linoleate, protective effect against x-radiation 141
— sex and 104, 145, 180
9,11-Linoleic acid, absence of biopotency in fat deficiency 103
Linoleic acid, biopotency of 101, 102
— — — — in fat deficiency 102
— — biotin-like effect on bacterial growth 135
— — growth requirement for lower organisms 137, 138
— — hepatic activity and 111
— — — — — choline oxidation 111
— — in choline metabolism 111
— — pyridoxine deficiency and 128
Linolenate, growth requirements 104, 105
Linolenic acid, biopotency of 101, 102
— — — — in fat deficiency 102
— — biotin-like effect on bacterial growth 135
Linoleyl alcohol, biopotency in fat deficiency 102
Lipolytic Theory (Verzár) 161, 162
Liver, functions of 142
— lipid composition, sex differences in 144
— regeneration following partial hepatectomy 143

METHIONINE and nitrogen excretion, effect of dietary fat on 112, 113
Methyl oleate, chlorophyll photo-oxidation of 86
Methyl linoleate, chlorophyll photo-oxidation of 86
Mineral oil, effect on utilization of carotene 131
— — — — — of fat soluble vitamins 131
Monoglycerides, lowering of interfacial tension by 208
— polymorphism of 29, 45
Monoiodoacetic acid, as an inhibitor of carbohydrate absorption 166
— — as an inhibitor of fat absorption 166
Monolayers 214
— area per chain 221
— at the surface of water 214
— changes of state in 216
— changes of state in (nomenclature) 224
Monolayers, chemical reactions in 232
— higher order transformations in 225
— interactions in mixed 230
— interfacial pressure π 213
— molecular area 221
— molecular area and molecular constitution 226
— penetration of 231
— phase transformation by pressure variation 224
— relations between substrate and 228
— surface potential 216
— surface pressure π 216
— surface pressure-area isotherms 215, 216
— surface viscosity 216
— temperature of changes of state in 222
Monomeric diperoxides, structure of 85
"Multigeneration tests" to determine nutritional value of various fats 176
Multiple contact, continuous counter-current extraction, applications of 322
Multiple stage, classification of extraction apparatus 301
— — conditions of optimal operation 303
— — continuous counter-current extraction 298
— — continuous process equipment 302
— — laboratory models for 301, 302
— — schematic representations of systems of 301

NATURAL oils, surface tension of 198
Neurospora crassa, growth requirements 137
— — — — — aspartic acid 137
— — — — — oleic acid 137
— — — — — Tween 80 137
Niacin, fat and 129
— synthesis of 129
Nitrogen, urinary, and fat intake 111, 112, 113, 114
10,13-Nonadecadienoic acid, absence of biopotency in fat deficiency 103

OCTADECENOIC acids, biotin-like effect 135

Octadecenoic acids, comparative biopotencies 134, 135
— — — — elaidic 134
— — — — oleic 134
— — — — petroselenic 134
— — — — vaccenic 134
Oleic acid, absence of biopotency in fat deficiency 102, 128
— — as substitute for biotin 133, 134
— — digestibility as related to glyceride structure 154
— — growth requirement for bacteria 133, 134, 135, 138
— — growth requirement for *Neurospora* 137
Oleomargarine, digestibility by man 146, 148
— digestibility by animals other than man 150
Oxidation, factors affecting rate of, antioxidants 80
— conjugation 73
— dilution 75
— "driers" 79
— free acids 74
— metal catalysis 79
— oxygen pressure 75
— peroxide catalysis 77
— physical state 84
— pro-oxidants 77
— refining procedures 82
— surface catalysis 80
— synergists 81
Oxidation, maximum rates of, acids and esters 73
Oxidation products, detection of 87
— — — — alkali colour 89
— — — — dicarbonyl compounds 89
— — — — ferric thiocyanate method 90
— — — — infrared analysis 91
— — — — iodometric method (peroxide number) 90
— — — — Kreis test 87
— — — — polarographic analysis 91
— — — — Stamm test 89
— — — — stannous chloride method 90
— — — — thiobabituric acid test 87
— — — — ultraviolet absorption 91
Oxidized fat, biological significance of 92
— — effect on enzyme systems 94
Oxidized fat, essential fatty acid destruction in 92
— — vitamin destruction in 92

Pantothenic acid, deficiency, dermatitis, and 127
— — — — — — fat as therapeutic agent 127
— — fat and 127
— — requirement for biosynthesis of fat 127
Partition Theory (Frazer) 161, 162
Peroxides, conjugation in 63
— histochemical detection of 90
— isomerism in 63
— non-conjugated 62, 86
— thermal decomposition of 56
— toxicity of 93
Phlorhizin, as an inhibitor of fat absorption 166
Phosphatides, adsorption at water-oil interface 213
2,6-Phytadienic acid, absence of biopotency in fat deficiency 103
2-Phytenic acid, absence of biopotency in fat deficiency 103
Polymerization of fats, effect on physiological and physical properties 155
— — — toxicity due to 178, 179
Polymorphism, of glycerides 1
— of saturated diglycerides 35
— of saturated monoglycerides 29
— of saturated triglycerides 1
— of unsaturated glycerides 39
Polyneuritis, fat intake and 125
Polyoxyethylene sorbitan monooleate (PSM) as emulsifying agent, effect on absorption of fats 165
Pregnancy, fat intake and 120, 121
— — — — animal *vs* vegetable fats 174
Protein, dietary, effect on digestibility of fats 156, 157
Protein metabolism, fat intake and 111, 112, 113, 114
Provitamin A (*see also* Carotene), comparative content in animal *vs* vegetable fats 167, 168
Provitamins D conversion to vitamin D by irradiation 169
PSM, *see* Polyoxyethylene sorbitan monooleate 165

Pyridoxine (vitamin B_6), fat and 127
— growth requirements 128

RADICAL systems, resonance energy associated with 62
Rancidity, meaning of 51
— of fats, in relation to tocopherol content 170
Reproduction, fat intake and 121
Riboflavin (vitamin B_2), fat and 126, 127
Ricinoleic acid, absence of biopotency in fat deficiency 102

SEX, as affecting requirements for essential fatty acids 104, 119, 120, 143
— as factor in fat utilization 143
Sexual maturity, fat and 119
Soaps, adsorption at the water-oil interface 210
Soap solutions, adsorption from (air-water interface) 235
Solid surface, multilayers on 230
Specific dynamic action, carbohydrate and 117, 118
— — — fat and 117, 118
— — — protein and 117, 118
Sprue, fat absorption in
— — — — effect of PSM 165
Stearic acid, digestibility as related to glyceride structure 154
Streptococcus faecalis, growth requirements 133
— — — — aspartic acid 133
— — — — unsaturated fatty acids 133
Stress factors, effect of dietary fat on response 139

TETRAHYDROXYSTERIC acid, absence of biopotency in fat deficiency 103
Thiamin deficiency, fat intake and 125
— — symptoms of 125
Thiamin metabolism, fat and 124, 125, 126
Thyrotoxicosis, dietary fat and 139, 140
— symptoms of, response to dietary fat 139, 140, 141
Tissue lipids, sex differences in composition 144
Tocopherol (*see also* Vitamin E), biotin sparing action 129
Tocopherol, protective effect in alloxan toxicosis 177
Triglycerides (*see also* Polymorphism *and* Glycerides)
— absorption rate 161, 162
— area per chain 221
— average coefficient of digestibility in animals 151
— digestibility as related to melting point 153
— — as related to structure 154
— parachor of 199
— surface tension of 196
Trihydroxystearic acid, absence of biopotency in fat deficiency 103
Tween 80 (*see* Polyoxyethylene sorbitan monooleate) 165

UNSATURATED fats, enzymatic oxidation with lipoxidase 86
Urea inclusion compounds, of fatty acids (*see also under* Fatty acids) 243

VACCENIC acid, in butter *vs* vegetable fats 172
— — effect on growth 172, 173
Vegetable oils, spectral characteristics of 82
Vitamin A, comparative content in animal *vs* vegetable fats 167, 168
Vitamin A, sources, animal tissues 168
— — butter 167
— — fish liver oils 168
— — margarine 168
— storage in liver, effect of diluent oils 130, 131
— utilization, as alcohol 129, 130, 131
— — as ester 129, 130, 131
Vitamin A, effect of diluent oils 130, 131
Vitamin B_1 (thiamin), fat and 124, 125, 126
Vitamin B_2 (riboflavin), requirement in relation to fat intake 126, 127
Vitamin B_6 (pyridoxine), deficiency symptoms 127
— — — — dermatitis 127
— — — — fat and 127, 128
Vitamin D, sources, animal fats 168, 169
— — by irradiation of provitamins D 169

Vitamin D, sources, fish liver oils 169
— utilization, effect of mineral oil ingested 131
Vitamin E, in animal and vegetable fats 169, 170
— physiological action. antioxidant effect 170
— — — antisterility effect 170
Vitamin K, utilization, effect of mineral oil ingested 131
— — effect of hydroxy fatty acids 131
Vitamins, fat soluble, fat and 129
— distribution in animal and vegetable fats 167
Vitreous state of glycerides 20

X-RADIATION injury, average survival time of rats 141
— protective effect of fats 141, 180
— sex differences in 145
X-ray data, of urea inclusion compounds 249
X-ray investigation of glycerides 20

PREFACE

In recent years interest in the chemistry and biology of lipid materials has grown rapidly. It is now realized that lipids, like proteins and carbohydrates, are not only important in the normal and abnormal behaviour of living matter, but also as raw materials for an ever increasing variety of edible and inedible manufactured products. Various substances have been included in the lipid classification, largely on the basis of biological interrelationships. From a purely chemical point of view lipids are a rather ill-defined and heterogenous group of substances. Nevertheless, even non-triglyceride lipids, whose biological importance has long been recognized, are now assuming commercial importance as materials for the production of food products, therapeutic agents and chemicals.

The reception given to Volume 1 of *Progress in the Chemistry of Fats and Other Lipids* has made it obvious that there is a need for the periodic publication of authoritative digests of recent advances in the more rapidly progressing areas of investigation. It appeared that this need could best be met by publication of a series of monographs containing the contributions of specialists in the areas of greatest interest. Because of the heterogeneity in this field, however, there was some question regarding division of subject matter. In the experience of the editors, the interests of lipid specialists extend into areas that may be quite unrelated to their own, so it was decided that these monographs should be published rather frequently, perhaps yearly, if warranted by the progress in lipids research, and should include timely chapters on all types of researches, whether biological, chemical, or industrial. The only area not included within the scope of this series is technology. The technology of lipid materials will be covered in a companion series of monographs entitled *Progress in the Technology of Fats*, the first volume of which will appear in the near future.

1

THE POLYMORPHISM OF GLYCERIDES

T. Malkin

DURING the past century, numerous workers have studied the interesting problem of the "double melting" of glycerides, and whilst it has long been known that this was due to polymorphism, the exact nature of this, and the relationship between the various polymorphic forms, was little understood. Indeed, as we now know, a clear explanation was hardly possible before the advent of X-ray diffraction methods. Once, however, that this new weapon had revealed the structure and also the general habit of polymorphism of simple long chain compounds (see Vol. 1, Ch. 1), the way was open for a new attack on this long-standing problem, and from 1934 onwards there appeared a series of thermal and X-ray studies of glycerides, by MALKIN and his collaborators, which developed the present views on glyceride polymorphism. More recently, DAUBERT, LUTTON, and other U.S. workers have joined MALKIN in this field.

The double melting of triglycerides

As far back as 1849, HEINTZ[1] observed that stearin, after melting and quickly solidifying, melted at 51–52°C, but on being heated further, resolidified and melted again at 62–62·5°C. A little later, DUFFY[2] gave, for his time, a remarkably clear account of the melting behaviour of tristearin, for which he reported three melting points, namely 52°, 64·2°, and 69·7°. He showed that the highest melting point was identical with that of the solvent crystallized glyceride, and that the lowest practically coincided with the setting point. He also noticed that the more he purified his tristearin, the more difficult it became to detect the intermediate melting point. This excellent work appears to have been overlooked by later investigators, and for many years only two melting points were reported for tristearin (BERTHELOT,[3] SCHEIJ,[4] GUTH,[5] LAUTZ[6]), for tripalmitin (MASKELINE[7]), and for trimyristin (REIMER and WILL[8]), thus giving rise to the well-knowr expression "double melting" of glycerides. In 1915, however, OTHMER[9] reported three melting points for tristearin and trimyristin, and a little later, LOSKIT[10] confirmed this, and also found three for tripalmitin. On the other hand, as recently as 1930, JOGLEKAR and WATSON,[11] in a careful study, reported only two melting points for a number of triglycerides of the highest degree of purity. Two years later, however, WEYGAND and GRUNTZIG[12] claimed the existence of seven different forms, for a number of triglycerides, as a result of a detailed microscopic examination. It should be observed, however, that visual observation is a notoriously unreliable method for the identification of different crystal forms, and hence these latter results must be regarded with some reserve.

The position therefore at this time, just prior to the first x-ray studies, was still very confused, and it is of interest to note that the views of DUFFY, eighty years earlier, most nearly summarized the position, namely, simple triglycerides exist in three modifications of different melting points, the modification of intermediate melting point often being difficult to detect.

Thermal and x-ray investigation

Briefly, the principle of this method is to establish the number and range of stability of the various polymorphs, by means of cooling and heating curves, and, on the basis of this, to determine differences in structure between the various forms, by x-ray examination.

MALKIN[13] had earlier used this method in a study of the dimorphism of ethyl esters of fatty acids, and there was good reason to expect equal success when it was applied to glycerides, since these are merely slightly more complex esters.

As expected, a close parallel was found. Ethyl esters had been shown to exist in two modifications, a stable form (β), in which the long chains are inclined with respect to the planes formed by the terminal methyl groups, and a less stable form (α), in which the chains are vertical and rotating. Both these forms were found to exist in the triglycerides,[14] namely, a stable β-form with inclined chains. and a monotropic α-form, with vertical chains. In harmony with views on alternation advanced earlier by MALKIN,[13] the melting points of the inclined β forms were found to alternate, whilst those of the vertical α-forms were non-alternating. The third and lowest melting form of the triglycerides, which is obtained by rapid cooling of the molten glyceride, was found not to be truly crystalline; it possesses many characteristics of a glass, and was therefore termed a vitreous form (γ).

On extending the investigation to mixed saturated glycerides (containing two different acid radicals), MALKIN and MEARA[15], [16] and CARTER and MALKIN[17], [18] found a fourth modification, with a melting point lying between those of the α- and β-forms. This they termed the β'-form, since it possessed non-rotating, tilted chains (see Vol. 1, p. 11, for distinction between α- and β-forms). On re-examination of the simple glycerides in the light of this discovery, the β'-form was found for those containing odd membered acids (see GRUNTZIG,[19] to whom these results were communicated). Unfortunately the work had to be discontinued at this stage, but some years later this form was also reported for simple even acid glycerides by a number of U.S. workers.[20], [21], [22]

Summarizing the position for saturated triglycerides in 1948, CLARKSON and MALKIN[23] stated that all the saturated glycerides that they had examined (some fifty), whether simple or mixed, exist in four solid forms, viz. in order of increasing melting point, vitreous, α- and β'- (crystalline, monotropic), and β- (stable crystalline) form. Except for those containing short chains, i.e. C_{10} and C_{12}, rapid cooling of the melt brings about solidification mainly in the vitreous form, which on warming passes through the following transitions vitreous →

$\alpha \rightarrow \beta' \rightarrow \beta$, and in some cases, vitreous $\rightarrow \alpha \rightarrow \beta$. These transitions are rapid for simple even acid, and symmetrical mixed triglycerides, but slow for simple odd acid, and unsymmetrical mixed triglycerides, the speed of transitions diminishing in all cases with increasing length of acid chain. It was shown that α-forms of simple even acid triglycerides may change directly into the stable β-form without passing through the intermediate β'-form, but the factors causing this somewhat exceptional transition could not be determined.

Cooling and heating curves

These play an important part in the investigation of glyceride polymorphism, and a clear understanding of the subject cannot be arrived at without a detailed study of such curves, taken under a variety of temperature gradients. The technique should preferably not be too elaborate, for it is desirable to take a number of curves for each glyceride, and the following simple method can be recommended: 0·75–1 g of the specimen is placed in a thin-walled test-tube of about $\frac{3}{8}$–$\frac{1}{2}$ in. diameter, and fixed by a cork in a tube of about three times this diameter to act as an air jacket. This is placed in a Dewar vessel, which may contain air, water, ice, or freezing mixtures, to give any desired cooling (or heating) gradient. A copper-constantan thermocouple, fixed centrally in the specimen, is attached to a Cambridge thread recording instrument, which marks a chart on a revolving drum every half-minute. The chart can be read to $\frac{1}{4}$°C, which is sufficient for most purposes, since all melting points can be checked by the standard capillary method.

Cooling curves

Although cooling curves differ in appearance considerably, according to the rate of cooling, there are, nevertheless, two distinct types, which correspond to the separation of α- and vitreous forms, respectively (Fig. 1). These are readily distinguished by the supercooling which almost invariably precedes α-crystallization. Supercooling cannot, of course, precede the separation of the vitreous form, for the temperature at which this occurs is essentially that below which the glyceride cannot remain liquid. Cooling curves normally give no indication of the separation of β'- and β-forms, and indeed, molten glycerides can be maintained for hours in the liquid state, at temperatures several degrees below the melting points of these forms. If, however, the melt is heavily seeded with β-form, or if it has not greatly exceeded in temperature the β-m.p. (i.e. there are still a number of minute β-seeds present), an arrest will appear at the β-m.p., but crystallization is excessively slow.

It appears, therefore, that whether β-seeds are present or not, the most facile route from the melt to the stable β-form is *via* the α-form, viz.:

$$\text{liquid} \rightarrow \alpha \begin{cases} \nearrow \beta \\ \searrow \beta' \rightarrow \beta \end{cases}$$

Crystallization in the α-form is, however, by no means rapid. For example,

JOGLEKAR and WATSON[11] showed that 1 g specimens of tristearin took from two to three hours to solidify at the α-solidifying point. It is clear, therefore, that the unwieldy glyceride molecules do not readily assume the regular layers required by the α-structure. Consequently, if a molten glyceride is cooled rapidly, solidification takes place, because of loss of molecular kinetic energy, before the required regularity of the α-form is achieved, and the form which has been termed vitreous separates.

Heat is evolved during this separation, for in an irregular manner, hydrocarbon chains and parts of chains are held together much as they are in a true crystal, and the "heat of crystallization" of the contacting methylene groups in adjacent chains is evolved. The main difference, therefore, between the crystal-

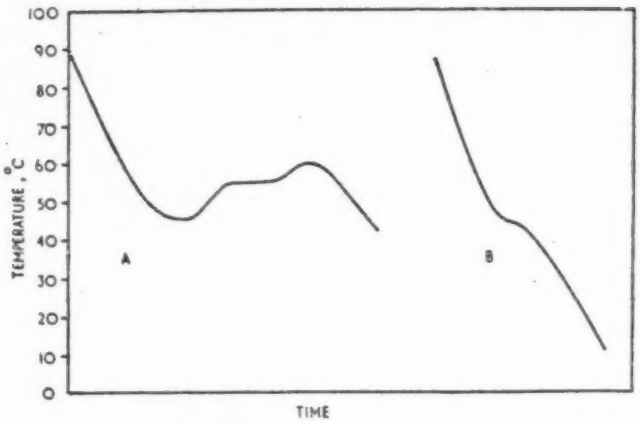


Fig. 1. Cooling curves for Tripalmitin.

line and the vitreous condition is that in the former, the regular arrangement permits the maximum number of methylene contacts, whereas in the latter, these are fewer, and may vary according to the rate of cooling.

The heat evolved during vitreous solidification is consequently less than that evolved during α-crystallization; moreover, because of the variable methylene contacts, the heat content of the vitreous solid varies, and hence the setting point (and also the m.p.) is not constant, but varies over a small range.

It will be clear from the above that the nature of the solid which separates, and the form of the cooling curve, will greatly depend on the rate of cooling; by varying this, solids may be obtained consisting of groups of molecules in all possible arrangements from the disorder of the liquid to the regular layers of the α-form, and even β′- and β-forms. A brief consideration of a few typical cooling curves will illustrate this.

Fig. 1A (tripalmitin, separation of α-form) is a typical curve, which can be obtained for most glycerides, if the cooling gradient is small. Considerable supercooling occurs, and the temperature may closely approach the setting point of the vitreous form before α-separation commences. At this point, the temperature rises and remains steady at the α-setting point for a short time,

after which there may be a further rise owing to partial transition into the β'- or β-forms, but the m.p. of the latter is not usually reached.

Fig. 1B is also a curve for tripalmitin, but with a greater cooling gradient. There is no supercooling and separation of vitreous form occurs, with relatively small heat evolution.

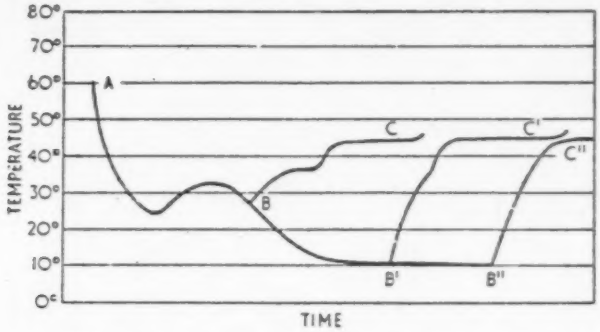


Fig. 2. Cooling and heating curves for Trilaurin.

Fig. 2 is a curve for trilaurin, showing α-separation: in this case, crystallization sets in at a temperature well above the vitreous setting point (15°C), but, nevertheless, only after considerable supercooling. The cooling gradient is too great to permit a steady temperature during α-separation, and therefore neither the maximum nor the minimum on this curve represents a definite setting point.

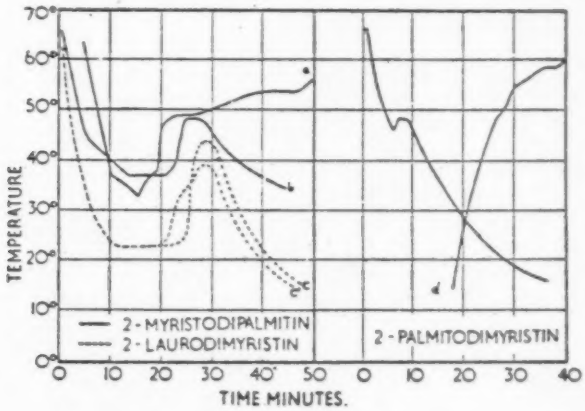


Fig. 3. Cooling and heating curves.

In a case of this kind, the α-m.p. can be determined by a heating curve (BC) or by adjusting the cooling gradient to obtain a steady arrest followed by a rise as in Fig. 1A. Fig. 3 *a*, *b*, are curves for 2-myristodipalmitin; the former, cooling to room temperature, shows separation of vitreous form, whilst the latter, cooled more slowly, shows first α-separation, followed by vitreous separation, and finally a rapid transition into β'- and β-forms. Fig. 3 *c*, *c'* (2-laurodimyristin) shows vitreous separation followed by transitions into α- and β'-forms.

The controlling factor in the separation of a crystalline solid from its melt is the rate of formation of crystal nuclei, and all the evidence from cooling curves of glycerides, shows that it is the rate of formation of nuclei of the α-form which determines the nature of the final solid. Formation of nuclei of β'- and β-forms,

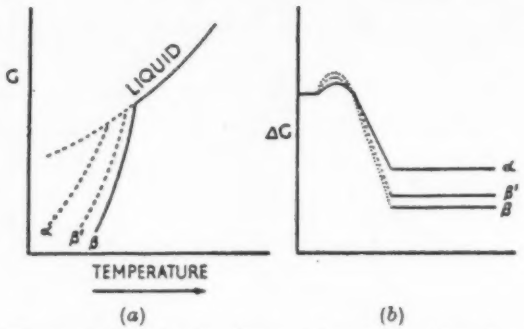


Fig. 4. (a) Free energies, (b) activation energies of nucleation of α-, β'- and β-forms of triglycerides (diagrammatic).

directly from the melt, is too slow to have any appreciable influence on the course of the solidification.

Below the melting point of the stable β-form, the liquid and the α- and β'-forms are metastable, i.e. their free energies (G) are higher than that of the

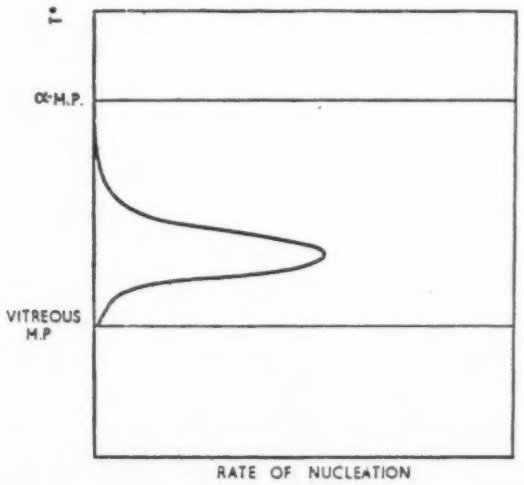


Fig. 5. Relation between temperature and rate of nucleation of α- forms of triglycerides (diagrammatic).

stable form (Fig. 4*a*). Nevertheless, crystallization does not commence spontaneously, for before this can begin, an energy barrier must be crossed, the activation energy of which, according to VOLMER,[24] is one-third of the surface energy of the newly formed nucleus. Fig. 4*b* illustrates this diagrammatically and indicates the greater difficulty of formation of β'- and β-nuclei.

It is seen from Fig. 4*a* that the greater the supercooling, the greater the free energy difference between the liquid and the α-form, and hence the greater the tendency to crystallize. On the other hand, increased supercooling results in increased viscosity, and hence the molecules cannot so readily move into positions favourable for nuclei formation. Consequently, somewhere between the α and the vitreous m.p. there will be a zone of optimum rate of nuclei formation, Fig. 5.

The position of this zone varies a little according to the nature of the glyceride, but in general it lies nearer to the vitreous than to the α-setting point.

This is illustrated by the sharp rise in temperature when specimens are maintained for any appreciable time in the neighbourhood of the vitreous setting point, cf. Fig. 3 *a*, *b*, *c*.

Heating curves

The type of heating curve obtained depends upon the previous cooling history of the specimen, and upon the heating gradient. If the molten glyceride is cooled rapidly, it passes through the zone of α-nucleation too quickly to form α-nuclei of the necessary critical size for further growth, although numerous embryonic nuclei (i.e. less than the above critical size) may be present. If a steep heating gradient is now applied to this solid, it melts at the vitreous m.p. and the embryonic nuclei break up and dissolve in the melt, as the temperature rises beyond the zone of rapid nuclei formation. The curve obtained shows a single slight arrest at the vitreous m.p.

With a smaller heating gradient, however, the embryonic nuclei may remain in the nucleation zone for a sufficient length of time to grow and initiate α-crystallization. The heating curve in this case would show a slightly larger arrest at the vitreous m.p., followed by a steep rise to a second arrest at the α-m.p.

In a similar way, too rapid heating may cause the dissolution of embryonic nuclei of β′- and β-forms, and, in general, the smallest practicable heating gradient should be employed.

As already indicated, if the original melt is submitted to a slower rate of cooling, the resulting solid may consist of vitreous and α-forms, α-form, or α- and β′-forms, and arrests will usually be found on the heating curves corresponding to the forms present. It should, however, be remembered that all the above forms are metastable, and they may change into higher melting forms when heated. These transitions take place the more rapidly the smaller the acyl chains, and the greater the symmetry of the molecule: they may indeed take place in the solid state at room temperature, as in the case with glycerides of lauric and lower acids. This is illustrated by the curves given in Fig. 2, where BC, B′C′, and B″C″ are heating curves taken at ½, 1, and 3 hr respectively, from the commencement of the cooling curve. The α-form, which first separates, slowly changes at room temperature into the stable β-form, as shown by the disappearance of the α-arrest from the heating curve. Typical heating curves are shown in Figs. 2*d* and 6.

Sufficient has now been said to indicate the value of the study of cooling and heating curves in the determination of the number and the stability of polymorphic forms. With the majority of triglycerides they readily reveal the existence of four solid forms, and it is only in the case of simple triglycerides of even acids where they fail to do so. Here the $\beta' \rightarrow \beta$ transition takes place rapidly in the solid state, during the course of the heating curve, and the β'-form does not actually melt; hence, there is no indication of this form on the curve.

Before leaving this section, it should again be strongly emphasized that without a detailed study of cooling and heating curves it is impossible to

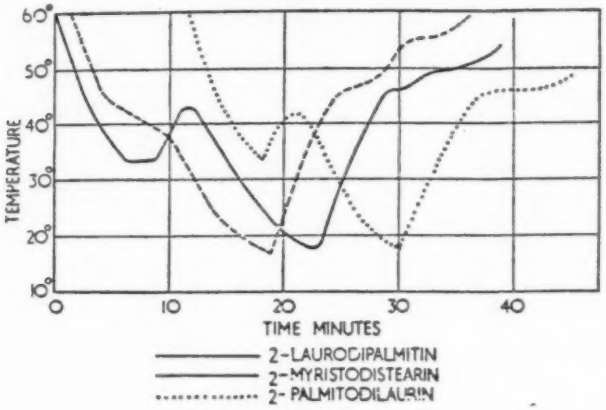


Fig. 6. Cooling and heating curves.

obtain a clear picture of glyceride polymorphism, and the failure of certain workers[25], [26], [27] to observe the forms found by MALKIN is due mainly to the neglect of these studies.

Capillary melting points

Early studies of glyceride polymorphism were carried out mainly by capillary m.p. determinations, and these afford useful confirmatory information to that obtained from curves. In some cases, the capillary method is the only means of determining the m.p., e.g. the β'-forms of simple even acid triglycerides.

There is no difficulty in the determination of the m.p. of the stable β-form, which is usually obtained by slow crystallization from non-polar solvents. The only precaution necessary is to raise the temperature very slowly in the region of the m.p., otherwise, owing to the slow melting of glycerides, it is easily possible to exceed the true m.p. by as much as a degree.

The determination of the m.p. of other forms requires a modified technique because of the possibility of transitions in the solid state, as the temperature of the specimen is being raised. Thus, the m.p. of the vitreous form is determined by immersing the capillary, containing the rapidly cooled glyceride, in a bath at a temperature at which melting and resolidification just takes place (vitreous $\rightarrow$ α-form). The capillary is now placed in a bath at a temperature at which the

α-form just melts and resolidifies (α → β′-form) and similarly for the m.p. of the β′-form. This, of course, involves a large number of repetitions, as the various bath temperatures must be found by trial, but the work is greatly reduced if the m.p.s of the different forms are known from heating curve studies.

The m.p.s are not quite so clearly defined when any particular form contains an appreciable number of nuclei of the higher melting forms. If, for example, a molten glyceride is cooled a little too slowly, the resulting vitreous form may contain numerous α-nuclei; on being immersed in a bath at the vitreous m.p., these nuclei grow rapidly as the vitreous form melts, and the mass appears to soften rather than melt, as it changes into the α-form. The same applies, of course, to α- and β′-m.p.s, which have frequently been reported as softening points. This is a sure indication that the particular specimens contained nuclei of higher melting forms.

These difficulties are partly due to the fact that solid glycerides are extremely bad conductors of heat, so that even when a capillary is immersed in a cold bath, the inner core of the specimen is protected by the solid outer shell, and may cool relatively slowly. Because of this, it is advisable to use thin walled capillaries of small diameter. As mentioned earlier, the capillary method affords the only means of determining β′-m.p.s of simple triglycerides of even acids, and even so, it is not possible to determine those of trilaurin and lower homologues, because of the speed of the transitions. The β′-m.p.s of higher members are determined as follows: The specimen is melted in a capillary, solidified in ice, and then placed in a bath one or two degrees above the m.p. of the vitreous form. Complete or nearly complete melting takes place, followed closely by resolidification in the α-form, but after some three to six minutes longer at this temperature, a further change takes place, denoted by a shrinking from the walls of the capillary. Before this change, the specimen melts almost completely at the α-m.p., but after the shrinking, the α-m.p. is passed without any change in appearance until the β′-m.p. is reached. At this point, softening and very rapid resolidification in the stable β-form takes place.

X-ray investigation

The x-ray technique has been described in some detail in Vol. 1, p. 8, and need not be further elaborated. Thin layers, either pressed or melted, and rods are examined in order to determine long and short spacings respectively.

In general, the determination of the spacings of the stable β-forms offers no difficulty, for this form can usually be obtained suitably crystalline by slow crystallization from solvents (preferably non-polar). Layers and rods of other forms are prepared by cooling thin melted layers or capillaries of the glycerides, followed where necessary by heating, under conditions determined by previous cooling and heating curve and capillary m.p. studies. From what has been said earlier, it will be appreciated that it is not always a simple matter to obtain the metastable forms entirely free from higher melting forms, and in order to obtain homogeneous specimens, repetition is often necessary. It must also be remembered that metastable forms of glycerides containing the shorter acids capric

and lauric may pass into stable forms at room temperature too quickly to permit the determination of x-ray data. In such cases, special arrangements for cooling the specimens during exposure must be made.

In order to obtain the vitreous form for x-ray work, a small amount of molten glyceride is spread on a microscope cover slip, in as thin a layer as possible, by means of a warm metal spatula. The layer is then cooled rapidly by holding the underside of the cover slip in ice-salt-water or solid CO_2-acetone, and the cover slip is then mounted on the spectrograph. Alternatively, the molten glyceride may be cooled in a fine capillary and ejected as a rod, although in this case, traces of α-form may be present owing to the relatively slower cooling in the centre of the rod.

These specimens may be converted into the α-form by holding them at the temperature of the vitreous m.p., or the specimens may be melted again and cooled less rapidly. Similarly, the α-forms may be converted near their m.p.s into the β'-forms. In certain cases, it is possible to obtain β'-forms by rapid crystallization from solvents, particularly ethanol or acetone.

With an x-ray tube running at about 15 mA, exposures of 20–30 minutes each side for layers, and 10–15 minutes for rods, are usually adequate, but sometimes melted layers, being not so well oriented, may require a little longer.

Long spacings

The long spacings of the various forms of simple triglycerides increase linearly with the carbon content of the constituent acids, and when plotted, fall on straight lines which cut the ordinate at C=O, in the neighbourhood of 4·5 Å, see Fig. 7.

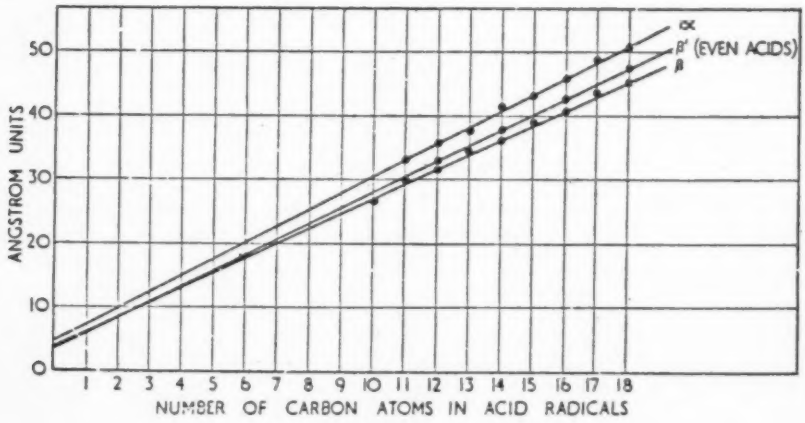
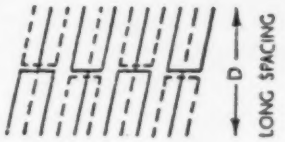


Fig. 7. Long spacings of simple triglycerides.

The spacings are too large to be related to the length of a single acid chain, and this led CLARKSON and MALKIN[14] to propose the tuning fork structure, where the spacing is related to twice the length of the acid chain, viz. ⊐—. Alternative molecules are regarded as lying head to tail across the reflecting

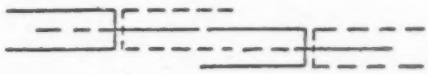
planes, either vertically (all α-forms and a few β′-forms) or tilted at a variety of angles (β′- and β-forms).



Since in Fig. 7 the spacings are plotted against the number of carbon atoms in the acid chains, the intercept at C=O (4·5 Å) is the contribution of the glycerol molecule and the terminal hydrogen atoms to the long spacing. The angle of tilt θ is given by $\frac{i}{1{\cdot}3} = \sin\theta$, where i is the increment of spacing per C atom in Å (1·3 Å is the maximum increment for a zig-zag chain of methylene groups, assuming the carbon atoms to be joined at an angle of 116°, see Vol. 1, Ch. 1).*

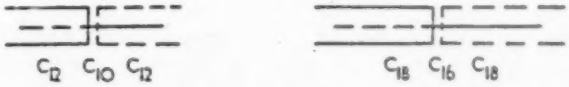
The above structure holds for all saturated mixed glycerides if the acids do not differ in length by more than two carbon atoms, but where they differ by four or more, there is usually a large increase in the long spacings. This led LUTTON[28] to propose the following triple chain length (T.C.L.) structures:

This latter group is of particular interest, for the α-forms and certain of the β′- and β-forms still appear to retain the previously described simpler structure. It may well be in these cases that there is some interpenetration of the chains, viz.:



Some light might be thrown on this problem by plotting the spacing against the effective length of the glyceride unit, and considering the intercept at C=O. For the simple structure of CLARKSON and MALKIN, the intercept is 4·5; any smaller intercept would indicate interpenetration of the chains, whilst the T.C.L. structure would give rise to an intercept of about 9 Å.

When plotting the long spacings of mixed triglycerides against carbon content, regularities can be expected only if structurally similar families are selected. Thus, the family of glycerides of acids $C_{12}C_{10}C_{12}$, $C_{14}C_{12}C_{14}$, $C_{16}C_{14}C_{16}$, $C_{18}C_{16}C_{18}$



have the same irregularity in structure at the terminal planes, i.e. the central chain is two carbon atoms shorter than the two outer, and the increase in total

* All angles of tilt given in this memoir are calculated on this assumption.

length from member to member is four carbon atoms. The long spacings of such a series would be expected to fall on a straight line, and hence the molecules would have the same angle of tilt across the reflecting planes. Similarly for the series $C_{10}C_{12}C_{10}$, $C_{12}C_{14}C_{12}$, $C_{14}C_{16}C_{14}$, $C_{16}C_{18}C_{16}$. On the other hand, a series such as $C_{18}C_{10}C_{18}$, $C_{18}C_{12}C_{18}$, $C_{18}C_{14}C_{18}$, $C_{18}C_{16}C_{18}$, $C_{18}C_{18}C_{18}$ is so markedly different from member to member that regularity in structure would not be expected, e.g.

greater interpenetration of chains is possible in *a* than *b*, and this might alter both the tilt and the actual length of the diffracting unit; as already mentioned, the irregularity in this group is so great that the first three members adopt an entirely different structure (T.C.L.). The effect of tilt on the long spacings appears to have been overlooked by some workers: thus, LUTTON[28] has proposed three straight line equations for the calculation of the α-, β'-, and β-forms of saturated triglycerides in general, on the assumption that each form has the same tilt as in simple triglycerides. This is definitely not so, as will be seen from Table 1, and it is therefore clearly impossible to calculate the spacings on the above simple basis. Again, BAILEY[29] finds it difficult to understand what configuration could give a greater long spacing for unsymmetrical dipalmitomyristin, than for tripalmitin. This is also clear from the tilts, for although the two molecules have approximately the same length, the angles of tilt are respectively 70° and 61°, and hence the spacing *D*, which is the distance between the reflecting planes, is greater for dipalmitomyristin.

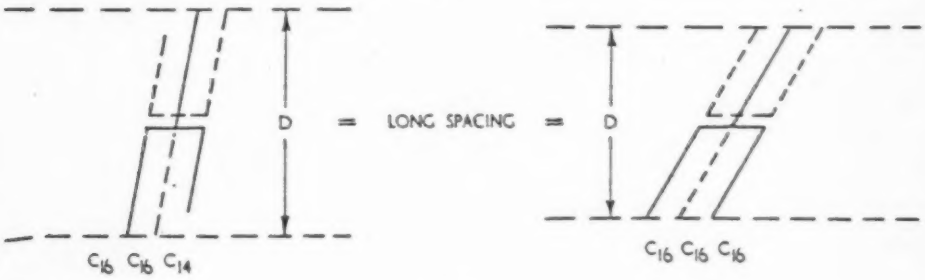


Table 1

	Angle of tilt, β′	*Angle of tilt, β*
Simple triglycerides . . .	67	61
Series $C_{12}C_{10}C_{12}$; $C_{14}C_{12}C_{14}$; etc. .	90	66
Series $C_{10}C_{12}C_{10}$; $C_{12}C_{14}C_{12}$; etc. .	79	66
Series $C_{10}C_{12}C_{12}$; $C_{12}C_{14}C_{14}$; etc. .	69	79
Series $C_{12}C_{10}C_{10}$; $C_{14}C_{12}C_{12}$; etc. .	65	66

Short spacings

Both the vitreous and the α-form give a single strong line at ≏ 4·19 Å, but the α-form is distinguished by the presence of sharp lines due to the long spacing. Owing to the difficulty indicated earlier, of avoiding the presence of small amounts of the α-form in the vitreous form, faint long spacings may appear on the photograph of a specimen which is predominantly vitreous, and this has led some workers to question the existence of the vitreous form.[20], [21] It should, however, be remembered that there is no sharp dividing line between the crystalline and non-crystalline state, and one visualizes a range of states from crystal → crystallite → embryonic crystallite → unit cell → disorder. With diminishing size of unit, x-ray diffraction becomes more and more diffuse and as the layer structure falls below a certain size, long spacings disappear. A diffused short spacing of ≏ 4·2 still persists, owing to lateral diffraction by hydrocarbon chains, which does not necessarily depend on a layer structure. A vitreous form, containing embryonic nuclei, might well give rise to weak long spacings, yet nevertheless melt at the vitreous m.p. The interpretation of x-ray photographs, in these cases, is therefore a matter of experience and judgement, the extreme cases being a single diffuse spacing of 4·2, accompanied by fogging due to general x-ray scattering, for a vitreous form, and a much clearer photograph showing sharp long and short spacings for an α-form. The α-form of glycerides has been so termed by analogy with α-forms of other long chain compounds which lie vertically across the reflecting planes (hydrocarbons, alcohols, ethyl esters) and which give rise to a single short spacing of 4·2 Å. Muller suggested that the chains in such compounds were rotating about their long axis. α-forms of glycerides are therefore vertical rotating forms, and since such forms are axially symmetrical, the existence of the so-called "tilted α-form" of Lutton,[30] is extremely improbable.

The β'- and β-forms give rise to groups of short spacings which can usually, but not invariably, be sharply differentiated. In general, the β-forms give lines somewhat widely separated, referred to as a "wide band," the strongest line of which is 4·6 Å. A typical wide band is that of the simple glycerides 3·7, 3·9, 4·6, 5·3 Å. The lines given by the β'-forms are usually closer together, and they have been termed a "narrow band," e.g. the β'-form of 2-decodilaurin gives 4·35, 4·16, 4·05, 3·82 Å, the strongest of which is 4·16 Å. Examples are given in Fig. 8.

The existence of the above three types of short spacings led Lutton[30] to make them the basis for nomenclature, thus

α-form — a single strong line ≏ 4·15 Å.

β'-form — usually two (but occasionally more) strong lines corresponding to ≏ 4·2 and 3·8.

β-form — a strong (usually strongest) line corresponding to ≏ 4·6 Å.

This has led to considerable confusion, for Malkin[15] had earlier introduced these terms to denote the polymorphs in order of increasing m.p.s viz. α, β', β. Lutton's terminology is unsatisfactory from many points of view, for as has already been shown both the vitreous and the α-forms give the single spacing of

4·19 Å; moreover, both the β'- and β-forms of trihydnocarpin, trichaulmoogrin and all the diglycerides give the strong 4·6 line which LUTTON ascribes to β-forms only, and finally, all the β-forms of the series of triglycerides $C_nC_{n+2}C_n$, $C_{n+2}C_{n+4}C_{n+2}$, etc., give the 4·2 and 3·8 lines. It would indeed be astonishing if the great variety of structurally different glycerides were to fall into the three clear-cut crystallographic groups suggested above.

LUTTON goes so far indeed, as to associate the various spacings with definite planes in the crystal, and ascribes (by inference from his diagrams, Fig. 9) monoclinic and triclinic structures to the β'- and β-forms respectively.

It will hardly be necessary to point out that it is quite impossible to deduce such structures on the basis of a few lines on a powder photograph. Whilst it is possible to identify planes in crystals of higher symmetry—cubic, hexagonal, trigonal, rhombohedral—on the basis of a large number of powder photograph

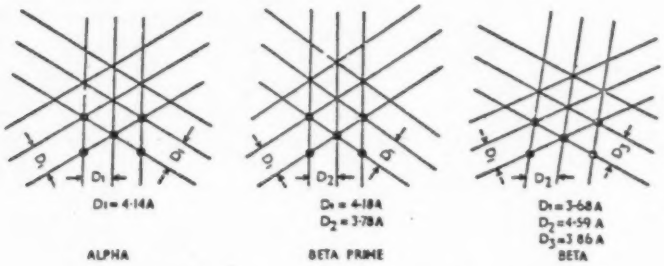


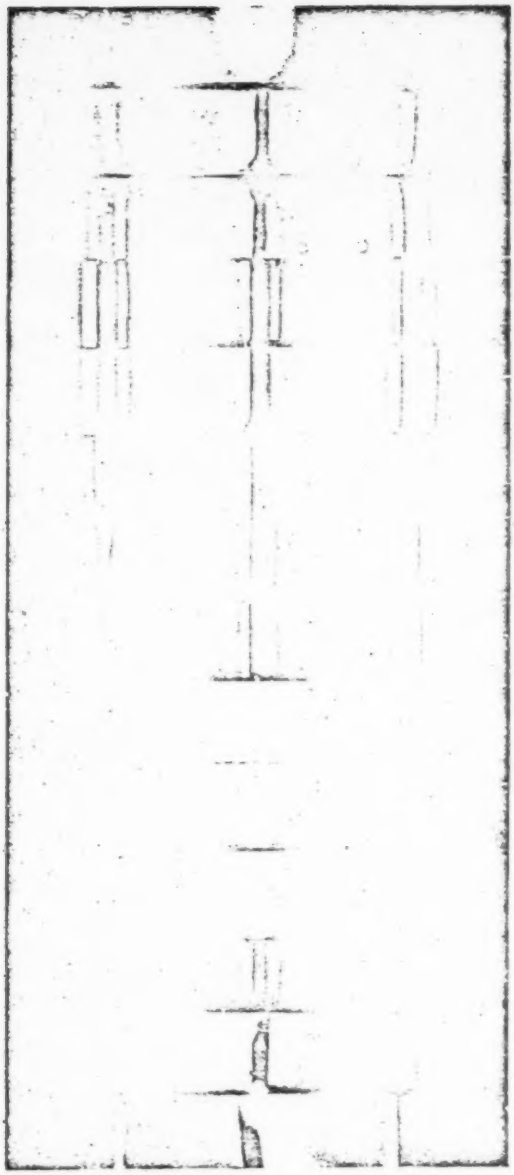
Fig. 9. LUTTON's suggested structures for glycerides.

lines, it is usually excessively difficult, or even impossible, to do so with orthorhombic, monoclinic and triclinic crystals. In the case of the latter, the necessary mathematical expression is so unwieldy that it is not worth while attempting to use it.

Planes of monoclinic crystals of certain long chain compounds can be identified from powder photographs, with a high degree of probability, where single crystal data are available, as is the case with hydrocarbons and fatty acids. No single crystal data are available for glycerides, however, and until these are forthcoming, any association of β'- and β-forms with definite crystal structures is pure speculation. FILER, Jr., SIDHU, CHEN, and DAUBERT,[31] who also find anomalies in LUTTON's terminology, are more circumspect, and rightly state that "judgement as to the positive association of specific and characteristic side-spacings (i.e. short spacings) for distinguishing the β- and β'-phases of triacid-triglycerides, must be reserved until additional information can be obtained . . ."

In the present article the terms α-, β'-, and β-forms are used, as has always been quite clearly stated by MALKIN, to denote the crystalline polymorphic forms in order of ascending melting points.

Before passing on to the detailed consideration of the various groups of triglycerides, the general picture may briefly be summarized. On slowly cooling the molten glycerides, considerable supercooling occurs and α-nuclei slowly form and initiate α-crystallization. This form consists of layers of molecules, lying



1-Monoglyceride, α changing to β′.

1-Monoglyceride, β′.

1-Monoglyceride, β.

a Type. 1, 3-Ditridecoin, β′.

b Type. 1, 3-Ditridecoin, β.

c Type. 1, 3-Dimyristin, β.

1, 2-Dipalmitin, α.

1, 2-Dilaurin, β.

Tripalmitin, β.

2-Myristodipalmitin, β.

2-Palmitodimyristin, β.

2-Oleodistearin, β″.

2-Oleodistearin, β′.

2-Oleodistearin, β.

Fig. 8. Typical short spacing of glycerides.

(To face page 14)

alternatively in head to tail position, vertically across the reflecting planes, with the long chains rotating freely about the long axis. The slow nucleation is due presumably to the requirement of a head to tail alignment, and to the increasing viscosity of the melt on cooling. If, at this stage, the heat of crystallization does not balance the external cooling, the chains become frozen in positions which on average, correspond to those of rotating chains, and the resulting α-form may be stable at room temperature for varying periods, according to the length of the chains. Such a solid gives long and short spacings corresponding to a hexagonal structure. If, however, the heat of crystallization balances or exceeds the external cooling, the molecules retain sufficient mobility to move into the more stable positions of the β'-structure. This in the majority of cases means that the chains lose their rotational energy and tilt across the reflecting planes (a few cases occur where the chains remain vertical). This $\alpha \rightarrow \beta'$-transition is probably a change from the hexagonal to a body-centred monoclinic form, a structure which is adopted by the majority of long chain compounds; but, as mentioned earlier, there is no direct evidence for this in the case of glycerides. Finally, the β'-form changes into the stable β-form at room temperature or near its melting point, and at different rates, according to the varying lengths of the acyl chains and the general symmetry of the molecule. In general, the $\beta' \rightarrow \beta$-transition is most likely a change into a slightly more stable monoclinic structure, similar perhaps to the change of the B → C forms in fatty acids, see Vol. 1, Ch. 1.

The picture of glyceride transitions is not so clear when the $\alpha \rightarrow \beta' \rightarrow \beta$ transitions involve large increases in the length of the spacings, i.e. formation of T.C.L. structures, and this group is worthy of a more detailed study, but the most urgent need in this field is single crystal data.

M.P. and X-ray Data for Saturated Triglycerides

Simple triglycerides

This group is the most complete homologous series of glycerides so far studied, and it exhibits the regularities in m.p. and x-ray data usually found in such series. Thus the m.p.s of the tilted β'- and β-forms alternate in a regular manner, whilst those of the vertical α-forms are non-alternating, Fig. 10. Again, the long spacings of tilted forms of odd and even members, plotted against the carbon content of the acyl chains, fall on different straight lines, Fig. 7, and the short spacings of odd and even members fall into separate, closely similar groups.

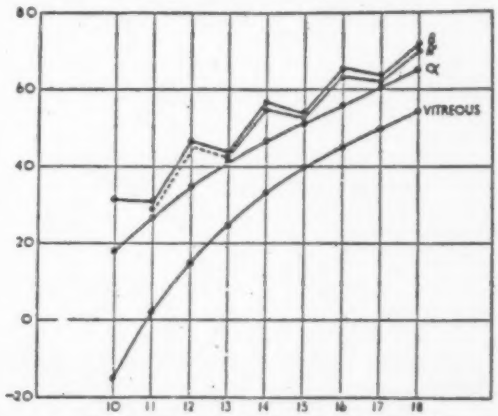


Fig. 10. Melting points of simple triglycerides.

Transitions of metastable

forms, at their m.p.s, into higher melting forms, are particularly rapid with the even acid members of this group, and it is not easy to obtain homogeneous α- and β′-forms. The even acid members also appear to be unique in passing directly from the α-form to the stable β-form, and these factors make the determination of the β′-m.p. difficult.

Transitions in the odd acid group, particularly the $\beta' \rightarrow \beta$ transition, are much slower, and it is rare to realize the β-m.p. arrest on a heating curve. No difficulty is experienced, therefore, in the determination of the m.p.s of the various forms of this group.

Table 2. Melting points of simple triglycerides
(CLARKSON and MALKIN)[23]

	Vitreous	α	β′	β
Tristearin . . .	54·5	65·0	70·0	72·0
Trimargarin . .	50·0	61·0	62·5	64·0
Tripalmitin . .	45·0	56·0	63·5	65·5
Tripentadecylin .	40·0	51·5	53·0	54·6
Trimyristin . .	33·0	46·5	54·5	57·9
Tritridecylin . .	25·0	41·0	42·5	44·0
Trilaurin . . .	15·0	35·0	—	46·4
Triundecylin . .	1·0	26·5	29·0	30·5
Tridecylin . . .	− 15·0	18·0	—	31·5

β′-melting points have not been determined for trilaurin and tridecylin.

Table 3. Long and short spacings of simple triglycerides
(CLARKSON *and* MALKIN)[13], [23]

	Long			*Short*		
	α	β′	β	α	β′	β
Tristearin . .	50·6	47·2	45·0	4·2	3.8s, 4·22s	3·7m, 3·9m, 4·6s, 5·3m
Trimargarin .	48·5	43·7	43·5	4·2	3·79s, 4·02w, 4·19s, 4·37w	3·65m, 4·0m, 4·6s, 5·3m
Tripalmitin .	45·6	42·6	40·6	4·2	3·8s, 4·22s	3·7m, 3·9m, 4·6s, 5·3m
Tripentadecylin .	42·9	39·1	38·9	4·2	3·81s, 4·12s, 4·3s	3·65m, 4·0m, 4·6s, 5·3m
Trimyristin .	41·2	37·6	35·8	4·2	3·8s, 4·22s	3·7m, 3·9m, 4·6s, 5·3m
Tritridecylin .	37·7	34·2	34·1	4·2	3·85m, 4·04w, 4·26s, 4·43w	3·65m, 4·0m, 4·6s, 5·3m
Trilaurin . .	35·6	32·85*	31·2	4·2	3·78s, 4·18vs*	3·7m, 3·9m, 4·6s, 5·3m
Triundecylin .	33·0	29·5	29·6	4·2	3·88s, 4·25s, 4·52w	3·65m, 4·0m, 4·6s, 5·3m
Tridecylin. .	—	—	26·8			3·7m, 3·9m, 4·6s, 5·3m

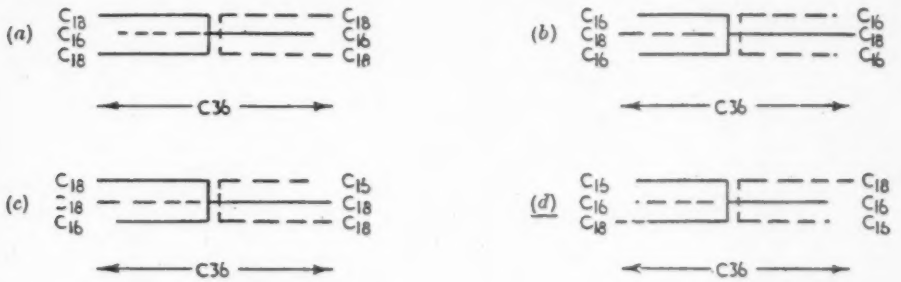
vs = very strong; s = strong; m = moderate; w = weak.
* LUTTON.[13]

Diacid triglycerides with acids differing by two carbon atoms

These fall into the following four groups in which the single acyl group is shorter or longer than the remaining two acyl groups by two carbon atoms.

Group (*a*) $C_{12}C_{10}C_{12}$, $C_{14}C_{12}C_{14}$, $C_{16}C_{14}C_{16}$, $C_{18}C_{16}C_{18}$.
Group (*b*) $C_{10}C_{12}C_{10}$, $C_{12}C_{14}C_{12}$, $C_{14}C_{16}C_{14}$, $C_{16}C_{18}C_{16}$.
Group (*c*) $C_{10}C_{12}C_{12}$, $C_{12}C_{14}C_{14}$, $C_{14}C_{16}C_{16}$, $C_{16}C_{18}C_{18}$.
Group (*d*) $C_{12}C_{10}C_{10}$, $C_{14}C_{12}C_{12}$, $C_{16}C_{14}C_{14}$, $C_{18}C_{16}C_{16}$.

Ir each group independently, the irregularity in structure due to the different lengths of the chains, is the same for each member, and since there is a uniform increase in the length of the molecule from member to member, regularities in m.p. and x-ray data are probable.



All the above glycerides exist in the vitreous, α-, β′-, and β-forms, the m.p.s of which fall on smooth curves for each of the above groups, Fig. 11, and the long spacings for each group show a linear relationship to carbon content. The

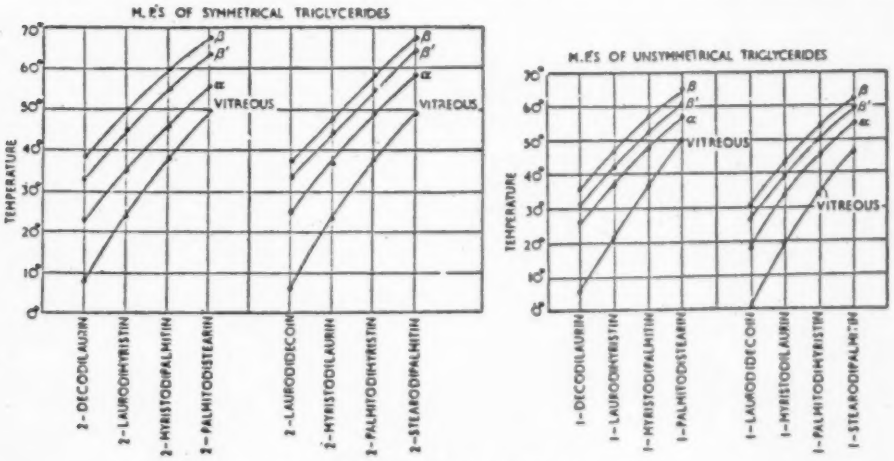


Fig. 11. Melting points of diacid triglycerides.

magnitude of the long spacings suggests the same structure as that of the simple triglycerides, i.e. a head to tail tuning fork structure, and if the spacings are plotted against twice the length of the longer acyl chain, the intercepts at C=O for (*a*), (*b*), (*c*), and (*d*) are respectively 2, 1, 2, 0, Å, which suggests some slight interpenetration of the chains.

Short spacings are similar to those of simple triglycerides and consist of broad and narrow bands for β- and β' forms respectively, except for the members of group (*b*), where both β'- and β-forms give rise to a narrow band of short spacings. By what appears to be more than a coincidence, the side spacings of the β-forms of the members of this group are practically identical with those of the β'-forms of simple odd acid glycerides of the same acyl length, e.g. trimargarin and 2-stearodipalmitin.

Table 4. Melting points of diacid triglycerides

	Symmetrical[15]				*Unsymmetrical*[17]			
	Vitreous	α	β′	β	*Vitreous*	α	β′	β
Decodilaurin	2	23	33	38·5	5	26	31	35·5
Laurodimyristin	24	35	45	50	22	37	42	46·5
Myristodipalmitin	37	46	55	60	36	47·5	52	57
Palmitodistearin	50	56	64	68	50	57	61	65
Laurodidecoin	6	25	34	37·5	0	17·5	26	30
Myristodilaurin	24	37	44	48	19	33·5	39	43·5
Palmitodimyristin	38	49	55	58·5	34	45·5	50·5	54
Stearodipalmitin	49	59	65	68	46·5	55	59·5	62·5

Table 5. Long and short spacings of diacid triglycerides[15], [17]

	Long			*Short*		
	α	β′	β	α	β′	β
2-Decodilaurin	—	—	30·0	—		3·79m, 4·06w, 4·35w, 4·62s 5·3m
2-Laurodimyristin	39·6	36·7	34·7	4·19	3·82s, 4·05w, 4·16s, 4·35w	3·84m, 3·89m, 4·61s, 5·3m
2-Myristodipalmitin	44·4	42·4	39·0	4·19	3·82s, 4·05w, 4·16s, 4·35w	3·74m, 3·86m, 4·61s, 5·3m
2-Palmitodistearin	50·5	47·5	44·2	4·19	3·82s, 4·05w, 4·16s, 4·35w	3·68m, 3·86m, 4·61s, 5·3m
2-Laurodidecoin	—	—	29	—		3·87s, 4·17m, 4·39s
2-Myristodilaurin	—	34·5	33·6	—	3·85s, 4·35*	3·86m, 4·06w, 4·26s, 4·45w
2-Palmitodimyristin	45·0	39·7	38·1	4·19	3·88s, 4·13s, 4·31s	3·81s, 4·13m, 4·31s
2-Stearodipalmitin	50·2	44·7	43·2	4·19	3·81s, 4·35*	3·81m, 4·03w, 4·2s, 4·48m
1-Decodilaurin		30·4	31·8	—	3·83, 4·18, 4·35	3·67m, 3·86m, 4·6s, 5·3m
1-Laurodimyristin		35·3	36·5	—	3·83, 4·18, 4·35	3·67m, 3·86m, 4·6s, 5·3m
1-Myristodipalmitin	43·9	40·3	41·5	4·19	3·83, 4·18, 4·35	3·67m, 3·86m, 4·6s, 5·3m
1-Palmitodistearin	48·8	44·7	46·5	4·19	3·83, 4·18, 4·35	3·67m, 3·86m, 4·6s, 5·3m
1-Laurodidecoin			28·4	—	3·83, 4·18, 4·35	3·75m, 3·86m, 4·6s, 5·3m
1-Myristodilaurin		34·5	33·0	—	3·83, 4·18, 4·35	3·75m, 3·86m, 4·6s, 5·3m
1-Palmitodimyristin	42·8	39·5	37·7	4·19	3·83, 4·22, 4·35	3·67m, 3·86m, 4·6s, 5·3m
1-Stearodipalmitin	47·8	43·9	4·25	4·19	3·83, 4·18, 4·35	3·67m, 3·86m, 4·6s, 5·3m

* = diffuse lines.

There is a marked difference in the speed of the transitions between the symmetrical (*a*) and (*b*) and the unsymmetrical (*c*) and (*d*) glycerides. With the former, the transition $\alpha \rightarrow \beta'$ is particularly rapid, and cooling curves of group (*a*) in particular show an unusually large rise in temperature, due to this change (see Fig. 3). This marked rise is not observed for any of the unsymmetrical glycerides, which are characterized by very slow transitions. As a consequence, capillary m.p.s of the various polymorphs of unsymmetrical glycerides are readily determined, for this group exhibits true "double melting," and no trouble is experienced with "softening" points. The transition $\beta' \rightarrow \beta$ is so slow in this group that even from solvents, the β'-form more commonly separates than the stable β-form. The latter is best obtained by extremely slow crystallization from non-polar solvents, preferably in a Dewar vessel. Unsymmetrical glycerides are easily distinguished, by their less crystalline appearance, from the symmetrical compounds, which form glistening masses of felted needles: under the microscope the former appear as short thickish prisms, and the latter as long slender, thin prisms. β'-forms have the outward appearance of precipitated chalk.

Diacid triglycerides (acids differing by more than two carbon atoms)

The polymorphism of this group is similar to that of other diacid triglycerides, the transitions being rapid for symmetrical and slow for unsymmetrical compounds. This is illustrated in the cooling curves for the symmetrical compounds, Fig. 6, which show a sharp rise in temperature as crystallization sets in, not observed on curves for the unsymmetrical compounds. The $\beta' \rightarrow \beta$ change for the latter group is exceedingly slow, and in the case of 1-laurodistearin, the β-form has not been obtained, although a m.p. comparison with the symmetrical isomer, suggests that it should exist.

Table 6. Melting points of diacid triglycerides

	Symmetrical[16]				*Unsymmetrical*[16]			
	Vitreous	α	β′	β	*Vitreous*	α	β′	β
Decodimyristin	16	37	40	43·5	15	32	38	43·5
Laurodipalmitin	34	47	50	53·5	32	45	49·5	54·0
Myristodistearin	47	56	59	62·5	44	54	57·5	62·0
Myristodidecoin	3	21	30	34	3	20	31	34·5
Palmitodilaurin	19*	35*	42·5*	45·5	20	33	43	46·5
Stearodimyristin	33	47	53	55·5	36	46	52	56
Decodipalmitin	20	42	48*	51·5	23	37	41	45·5
Laurodistearin	36	52	58	60·5	36	47	52	—
Palmitodidecoin	6	27	36	40	2	24	32	35
Stearodilaurin	21	38	43	47	20	31	41·5	45
Stearodidecoin	5*	34	40	44·5	13	32	38	41
Decodistearin	30*	47*	53*	57	33	42·5	46	49

* Difficult to determine owing to speed of transitions, probably accurate to 1°C.

When members of this group are arranged in appropriate families, e.g. $C_{14}C_{10}C_{14}$, $C_{16}C_{12}C_{16}$, $C_{18}C_{14}C_{18}$, they exhibit regularities in m.p. and x-ray data, but some divergences occur among the x-ray data, owing to possibilities of both the simple and the triple-chain-length structure, and a more detailed x-ray investigation will be required before some of these points can be cleared up. The greatest difficulty in this group, is to obtain a clear picture of the change from the simple to the T.C.L. structure by a mechanism not involving a large heat change since this would appear to involve the breaking and re-making of a large number of lateral methylene linkages. Moreover, the conditions under which glycerides of apparently similar structure adopt one or other of these structures, is not clearly understood, e.g. the β'-forms of 2-decodipalmitin and 2-laurodistearin.

Table 7. Long and short spacings of diacid triglycerides[16], [18]

	Long spacings			Side spacings									
	α	β′	β	β′				β					
2-Decodimyristin		33·7	52·5	3·92	4·15	4·39w		3·84	4·2w	4·6		5·25w	
2-Laurodipalmitin	44·6	77·0	59·0	3·81	4·08	4·36		3·84	4·2w	4·6		5·25w	
2-Myristodistearin	49·5	44·7	65·8	3·77	4·12	4·26		3·84	4·2w	4·6		5·25w	
2-Myristodidecoin	—	30·3	46·5	3·9	4·3w	4·6w		3·86		4·6	4·96w	5·29w	
2-Palmitodilaurin	—	36·6	35·5	3·9w	4·09w	4·29	4·44	3·85	4·09w	4·29	4·44w		
2-Stearodimyristin	44·0	41·0	40·0	3·88w	4·17	4·34		3·82	4·17	4·34			
2-Decodipalmitin	39·0	74·0	56·5	3·82	4·08	4·31		5·84		4·61		5·32w	
2-Laurodistearin	47·1	42·4	63·7	3·82	4·08	4·31		3·84		4·61		5·32w	
2-Palmitodidecoin	—	—	49·5					3·85	4·3vw	4·58	4·8vw	5·27w	
2-Stearodilaurin	40·8	37·5	56·8	3·83	4·02w	4·2	4·41w	3·85	4·3vw	4·58	4·8vw	5·27w	
2-Stearodidecoin	—	—	51·6					3·87	4·24w	4·58	4·9w	5·29w	
2-Decodistearin	—	76·3	61·2	3·83	4·11	4·34		3·86		4·61		5·34	
1-Decodimyristin	—	33·8	35·2	3·8	4·12	4·33		3·72	3·9	4·53	4·67	5·4	
1-Laurodipalmitin	45·4	38·5	39·8	3·78	4·09w	4·26	4·5w	3·72	3·9	4·53	4·67	5·4	
1-Myristodistearin	48·5	43·4	45·0	3·78	4·09w	4·26	4·5w	3·72	3·9	4·53	4·67	5·4	
1-Myristodidecoin	—	31·3	47·5	3·8	4·1w	4·26		3·84		4·6	4·84w	5·21w	
1-Palmitodilaurin	—	36·2	54·6	3·78	5·09w	4·26		3·84		4·6	4·84w	5·21w	
1-Stearodimyristin	46·4	41·7	61·4	3·8	4·15	4·27		3·84		4·6	4·84w	5·21w	
1-Decodipalmitin	—	74·1	56·2	3·82	4·1	4·35		3·84	4·27w	4·6	4·85w	5·26w	
1-Laurodistearin	47·4	42·8	—	3·82	4·18	4·35							
1-Palmitodidecoin	—	—	49·7	3·94		4·35		3·84	4·27w	4·6	4·85w	5·26w	
1-Stearodilaurin	—	38·7	57·0	3·86	4·11	4·35		3·84	4·27w	4·6	4·85w	5·26w	
1-Stearodidecoin	—	51·0	52·6	3·8	4·1	4·35		3·84		4·6		5·3	
1-Decodistearin	73·7*	—	60·0					3·74	4·02	4·33	4·6w		

All α-forms give a single spacing 4·19 A. w = weak lines.
* Possibly β'.

The vitreous form of triglycerides

With the exception of a few additional data of CLARKSON and MALKIN[23] in 1948, those so far discussed are due to MALKIN *et al.* up to 1939, and they lead to the satisfactory and harmonious explanation of the polymorphism of triglycerides which was summarized on pp. 2, 3. In 1945, however, LUTTON[20] and

other American workers,[21], [22] none of whom had had any previous experience in this difficult field, advanced the view, on the flimsiest of evidence, "that the concept of the glassy state of triglycerides should be eliminated" and that therefore triglycerides exist in only three solid modifications. But since MALKIN *et al.*, over the previous dozen years, had published m.p. data for some forty triglycerides, all existing in four forms, this new suggestion has naturally given rise to some confusion, which has not been lessened by LUTTON's misuse and misapplication of MALKIN's terminology (see p. 13). Nor were matters improved when LUTTON,[28], [32] who for long insisted on the existence of only three forms, began at last to find four, and was consequently compelled to introduce a still more confusing terminology. This view has retarded progress so greatly that it has become an urgent matter to settle the question finally, and in order to do so LUTTON's view will now be examined a little more closely.

There is, of course, nothing inherently improbable in the view that glycerides may exist in a vitreous form. TAMMANN, one of the greatest authorities on the phenomena of melting and crystallization, expressed the view that most substances could probably be obtained in the vitreous form. To illustrate this he took, more or less haphazardly, as many organic substances as he could find about his laboratory (153), and melted and cooled them at different rates, and found that 59 could exist as glasses.[33]

Many of the early workers recognized that the lowest form of glycerides was non-crystalline, thus HEINTZ[1] speaks of tristearin cooling to a transparent porcelain-like mass, and the same expression was used by REIMER and WILL[8] for trimyristin. LOSKIT[10] stated that the lower glycerides solidify as glasses, whilst GUTH[5] speaks of an amorphous labile condition. Quite recently, RAVICH, ZURINOV, VOLNOVA, and PETROV,[34] in a thoughtful paper, which shows a clear understanding of the principles of glyceride crystallization, adduce optical and other evidence for the existence of the vitreous form of glycerides. Part of their summary is worthy of quotation. "A thin layer of molten trilaurin between coverglass and slide, upon being cooled off at a given rate (by placing it on a cooling stage of temperature 4°C) yielded the transparent vitreous γ-form, which does not reveal anisotropy on microscopic examination in polarized light. This form is exceedingly brittle, shatters into fragments at a blow and upon softening assumes a salve-like consistency. Upon extremely rapid cooling of trilaurin melts (by immersing the samples in liquid air) they acquire the property of double refraction, like compressed glass plates." Fig. 12 taken from the above paper, shows the curiously cracked appearance of the solidified trilaurin referred to, and the shattering effect when this is struck.

In a recent paper from LUTTON's laboratory, QUIMBY[35] also refers to the rigidity and brittleness of the lowest melting form, which he finds fractures under high pressure. Now it is fairly common knowledge that the most characteristic property of long chain compounds in general is their softness and "soapy" feel. This is due to the regular layers of weakly attracting methyl groups in the crystal, which form the main cleavage planes: the fact that the above low melting forms fracture and shatter is the strongest evidence that these planes

are absent, and that the solid is not truly crystalline. QUIMBY, who also considers that the vitreous form does not exist, refers frequently to the well-known spherulitic formations which are so characteristic of glycerides, but he does not seem to have considered the implications of this. Spherulitic growth is a direct consequence of crystal growth in a highly viscous melt. Practically all the naturally occurring spherulites are known to have been formed in rapidly cooled viscous magmas, and they are common in rock glasses whose chief components are alkali feldspars and quartz. Artificial spherulitic structures often develop in badly annealed glass. In short, the formation of spherulites is diagnostic of a highly viscous melt, and it can safely be predicted that a substance which gives rise to spherulites will always form a glass when its melt is cooled quickly. As is well known, the ability of a substance to supercool and finally form a rigid glass can roughly be related to its viscosity, or to the activation energy constant (B) in the relation between viscosity and temperature $\eta = Ae^{B/RT}$. A high value of B means that the molecules cannot easily change their positions, and this is clearly a measure of resistance to crystallization: it also means a high rate of increase in viscosity with falling temperature, and hence a high probability of forming a rigid glass at some lower temperature. The striking resistance to crystallization of glycerides has been stressed earlier (pp. 3, 4).

It will be of interest to now consider LUTTON'S views more fully. According to him, most glycerides exist in three forms, and there is no vitreous form. It was, however, necessary for him to explain the data in Tables 4 and 6, which give the m.p.s of four forms for some forty glycerides. LUTTON therefore concluded that MALKIN and his collaborators had completely misinterpreted their results, and that their vitreous, α- and β-forms were really α-, β'-, and β-forms respectively: moreover, all the β'-m.p.s given in the above tables were fictitious, since owing to a remarkable and extremely convenient effect known as "degree of stabilization," β-m.p.s of pure glycerides may vary over a range of anything up to 5°. This latter view is too naïve to warrant discussion, but it will be worth while to consider the β'- and β-m.p.s a little further. Crystallographically, the β'- and β-forms are similar in that they possess tilted, non-rotating chains, and they are also probably both monoclinic. It is not likely therefore that their free energy difference, nor their difference in m.p. will be great. From the previous tables, the average m.p. differences are:

Simple even glycerides, 2·2°; mixed glycerides (Table 4), 4·1° (Table 6), 3·5°, and for a further large group of mixed triglycerides investigated by CHEN and DAUBERT[36] (Table 11), 3·3°, the extreme limits of difference being 1·2° and 5·5°, and it may be mentioned, in passing, that the differences between the β'- and β-m.p.s of mono- and diglycerides are of the same order.

The differences between β' and β-m.p.s found by LUTTON[20], [30], [32] are as follows:

PSS, 0·2; PSP, 1·0; SSS, 9·1; PPP, 10·0; MMM, 9·9; LLL, 10·0; PMP, 13·3; SMM, 9·9; PMM, 7·9; SLL, 13·6; where L = lauric; M = myristic; P = palmitic; S = stearic; PSS = palmitodistearin, etc.

With the exception of the first two examples, these differences are of an

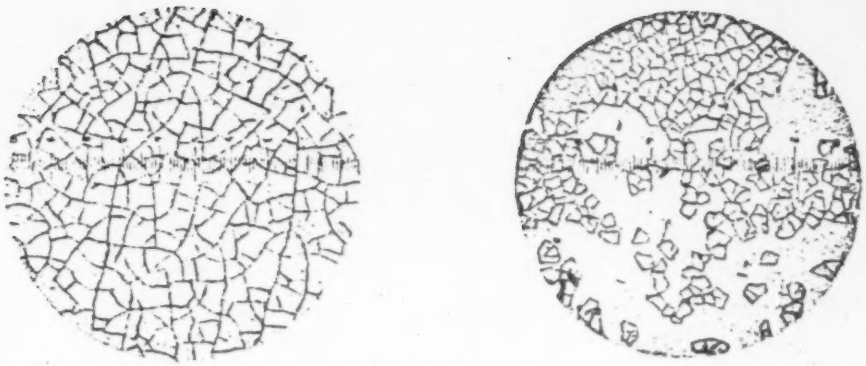


Fig. 12. The vitreous form of trilaurin.

(To face page 22)

entirely different order from those of MALKIN, and CHEN and DAUBERT, and they clearly refer to a difference between the α and β m.p.s. This would be expected to be larger, because of the high energy content of the vertical rotating chains. In other words, LUTTON's β′-form is in fact the α-form as MALKIN originally stated. That this is so, is also clear from a consideration of the simple triglycerides. Both LUTTON and MALKIN agree that the β′- and β-forms are tilted. Hence the melting points of these forms should alternate, for there is no known exception to the rule that tilted forms alternate (see Vol. 1, p. 15). According to LUTTON, however, the β′-m.p.s fall on the smooth curve marked α in Fig. 10.

Before concluding this section, a little should be said concerning LUTTON's interpretation of cooling and heating curves. In only one case, that of 2-stearo-

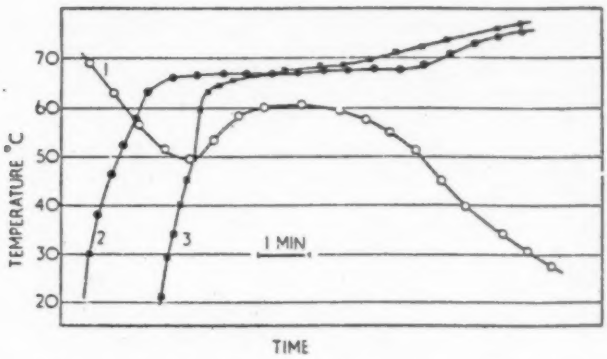


Fig. 13. Cooling and heating curves for 2-stearodipalmitin (LUTTON).

dipalmitin, has he carried out any systematic study of curves, and these were taken too rapidly to be of any real value. LUTTON considers that only two forms exist for this glyceride, namely the α-form of m.p. 46·5 and the stable form of m.p. 68·6° which he wrongly terms the β′-form, contrary to MALKIN's original definition. On the other hand, MALKIN and MEARA[15] found vitreous, α-, β′-, and β-forms, melting respectively at 49, 59, 65, and 68°C.

LUTTON could give no explanation whatever for curve 1, Fig. 13, reproduced from his paper, for neither arrest corresponds with either of his stated m.p.s; it is, however, a typical curve showing separation of α-form, similar to those discussed on pp. 4, 5. After supercooling, almost to the vitreous set point, which, as has been pointed out, is often the region of maximum α-nucleation, crystallization of the α-form sets in, and the curve rises to the α-m.p. In this region, some $\alpha \rightarrow \beta'$ transition takes place, but the heat of crystallization and transition is not sufficient to overcome the external cooling and the temperature slowly falls. With a slightly smaller cooling gradient, a second rise would almost certainly appear on this cooling curve.

The heating curve 2, does in fact show a slight inflexion at the α-m.p. (59°), but as the entire rise in temperature to the main m.p. occupied barely a minute

and a half, it is not surprising that this arrest is so slight, and that it was overlooked by LUTTON. In both of the heating curves the melting begins at about 65° (the β'-m.p.s of MALKIN and MEARA) and at no point is there a flat portion at 68·6°, the m.p. claimed by LUTTON. Clearly, this arrest is due mainly to the melting of the β'-form which is accompanied by a small amount of $\beta' \rightarrow \beta$ transition, but at no time is there any appreciable amount of the β-form present. In a later paper, LUTTON[30] gives a schematic cooling curve for tristearin (Fig. 14), showing the forms separating at the different points of the curve. This is the ideal curve for all glycerides discussed earlier, except that the arrests marked β' and β should be α and β'.

If LUTTON's interpretation of the curve is applied to the case of PSP, first considered, it will be seen that the α-form is crystallizing out some degrees

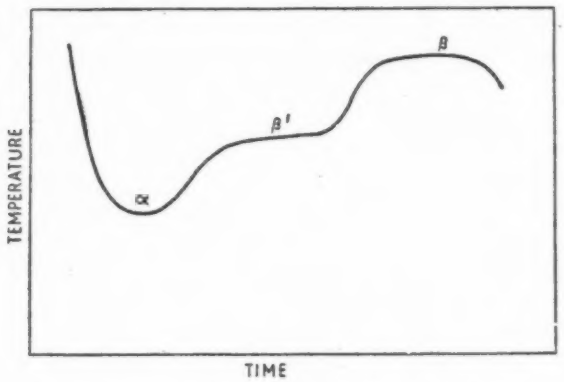


Fig. 14. Schematic cooling curve for tristearin (LUTTON).

above its m.p. A consideration of the heats of crystallization of the various forms shows that LUTTON's interpretation of the above curve is wholly untenable. KING and GARNER[37] have shown that the heats of crystallization per methylene group for α- and β-forms is 0·708 calories and 1·04 calories respectively, which agrees reasonably well with the ratio of the heats of fusion determined by CHARBONNET and SINGLETON[38] for α and β tristearin, namely 38·9 and 54·5 calories per gram, i.e. 38·9 for α formation and 15·6 for $\alpha \rightarrow \beta' \rightarrow \beta$ transitions. In other words, 70% of the heat evolved during crystallization is due to α-formation. According to Fig. 14 the heat evolution during α-formation is not more than 10% of the whole.

The same point is demonstrated by the steep rise characteristic of the heating curve when the vitreous form melts (Fig. 13; cf. also Fig. 3). If the vitreous form were the α-form as LUTTON suggests, the only heat available to produce this rise would be the relatively small heat of transition $\alpha \rightarrow \beta'$, for by far the larger part of the heat of crystallization would have already been evolved during the crystallization of the α-form. Quite clearly, the explanation is that LUTTON's so-called α-form is vitreous, and the steep rise in temperature is due to the delayed crystallization of the α-form.

Sufficient has now been said to show that the view that there is no vitreous form leads to contradictions and errors from whatever angle it is approached. Its introduction has caused the greatest confusion, and in the interests of the subject it should be discarded.

Further diacid triglycerides

JACKSON and LUTTON[39] have reported the m.p. and X-ray data for behenyl-palmitic and behenyl-stearic glycerides given in Tables 8 and 9. Their lowest melting forms are probably vitreous, and to preserve a uniform terminology,

Table 8. Melting points of behenyl mixed triglycerides

	PBP	*SBS*	*PBB*	*SBB*
Vitreous . .	47·4	56·0	55·9	61·3
β' . . .	61·5	64·0	66·1	71·5
β . . .	66·6	70·6	—	73·5

Table 9. Long and short spacings of behenyl mixed triglycerides

	PBP	*SBS*	*PBB*	*SBB*
Sub-alpha . .			58·9	61·4*
Vitreous . .	50·0	53·2	57·4	59·1*
β' . . .	46·0	49·4	52·4	53·3
β . . .	70·4	75·1	—	54·6
Sub-alpha . .			4·2s	4·2s
			3·72m	3·77m
Vitreous . .	4·14vs	4·14vs	4·15vs	4·15vs
β' . . .	4·19vs	4·18vs	4·20vs	4·20vs
	3·76s	3·78s	3·78s	3·78s
β . . .	4·58s	4·58s		4·59s
	3·79vs	3·78vs		3·87s
				3·70s

* It is doubtful whether this difference in the long spacings of the sub-alpha and vitreous forms is real: only two orders were found for the former d = 62·5S and 60·3, and therefore the experimental error is of a high order.

they have been so designated in the table. The authors report the discovery of a new low temperature form, termed sub-alpha, but no m.p.s were found for these forms, and the only evidence for their existence is the appearance of a short spacing in the region of 3·7–3·9 Å, at low temperatures (— 50°). At such low temperatures, however, ice condenses on the specimen, and gives rise to a spacing in this region, and it would be unwise to accept the existence of the sub-alpha form on this evidence alone.

A later paper by JACKSON, WILLE, and LUTTON[40] gives m.p. and X-ray data for 2-acetyl, 2-butyryl, and 2-caproyl distearin and dipalmitin (Table 10).

Although six different polymorphic forms are claimed to exist in this group, none exhibits more than three m.p.s, and a critical examination of the X-ray data suggests that only the α- and the stable β-forms are definitely characterized.

Table 10. Thermal and x-ray data of acetyl, butyryl, and caproyl diglycerides[40]

	SC_2S	SC_2S PC_2P	PC_2P	SC_4S	SC_4S PC_4P	PC_4P	SC_6S	SC_6S PC_6P	PC_6P
	Melting points, °C								
Sub-alpha	40·4		28·4						13·4
Alpha				33·4		20·6	27·7		
Super-alpha				42·7		32·6			
Sub-A-beta							46·0		40·5
Sub-B-beta							46·0		40·5
Beta	62·8		54·8	54·8		46·5	53·1		44·5
	X-ray data: long spacings, Å								
Sub-alpha-1 (-3)	28·2		25·7	29·8		26·9	30·4		53·7
Alpha-1				28·5		25·9	30·0		
Super-alpha-1				26·6		24·2			
Sub-A-beta-3							55·6		52·8
Sub-B-beta-3							54·5		49·2
Beta-3	52·1		47·2	48·4		45·0	56·8		52·5
	Short spacings, Å								
Sub-alpha		4·20s			4·15vs			4·20vs	
		3·78m			3·71m			3·70m	
		2·50vw							
Alpha					4·14s		4·15vs		
					2·44vw		2·50vw		
					15·75s*				
Super-alpha					5·00w				
					4·14vs				
					2·43vw				
Sub-A-beta								5·15m	
								4·69s	
								4·55s	
								3·86s +	
								3·55m −	
Sub-B-beta								5·38m	
								4·59vs	
								3·87s	
								3·66s	
Beta		4·59s			4·60vs			5·19m	
		3·87m			4·04s			4·61vs	
		3·72vs			3·85s			3·82vs	
					3·69m				
					3·54m				

* This is obviously not a short spacing.

The authors' description of the behaviour of SC_4S and PC_4P is of considerable interest and is worthy of quotation. "The super-alpha form, observed for SC_4S, and PC_4P, is unique thus far for triglycerides. The name was chosen because the new polymorphic form had the characteristic 4·14 Å short spacing of alpha while its melting point was 10–12° higher than that of alpha———. The super-alpha form was obtained by tempering the alpha form at 0·5° below its melting point for 2–4 minutes." Thus the authors unwittingly confirm MALKIN's view as to the nature of their alpha form. As was pointed out earlier, the lowest melting forms are vitreous, but they often contain sufficient crystallites of the α-form to give an X-ray pattern of this form. When this form is held near its m.p. it changes into the higher melting α-form (LUTTON's super-alpha) which naturally gives the spacings of the α-form. Far from being unique, this is the general behaviour of all triglycerides.

Triacid triglycerides

CHEN and DAUBERT[36] and SIDHU and DAUBERT[41] have determined the m.p. data for a series of triacid triglycerides, given in Table 11. It is not clear how

Table 11. Melting points of triacid triglycerides[36], [41]

	I	II	III
C_{18}—C_{14}—C_{16}	59·5	56·1	40·6
C_{18}—C_{12}—C_{16}	57·5	56·0	32·0
C_{18}—C_{10}—C_{18}	55·0	53·8	20·1
C_{18}—C_{16}—C_{14}	58·5(59·0)	56·0(54·0)	40·3(43·5–44)
C_{18}—C_{12}—C_{14}	55·0	51·9	28·8
C_{18}—C_{10}—C_{14}	52·5	50·1	14·0
C_{18}—C_{16}—C_{12}	52·0	47·0	33·4
C_{18}—C_{14}—C_{12}	49·5	45·5	27·5
C_{18}—C_{10}—C_{12}	—	41·8	22·3
C_{18}—C_{16}—C_{10}	50·0	46·5	26·1
C_{18}—C_{14}—C_{10}	45·0	42·0	21·5
C_{18}—C_{12}—C_{10}	44·0	40·0	14·5
C_{16}—C_{14}—C_{12}	49·0	44·0	37·0
C_{14}—C_{12}—C_{10}	37·0	34·0	22·0

far the glycerides were investigated by cooling and heating curves, for no details are given; but m.p.s are given for three forms termed I, II, III. CHEN and DAUBERT made the interesting observation that several members of the group could be obtained in form II, by crystallization from solvents, whereas, of course, the stable form I would be expected, and in a later paper, FILER, Jr., SIDHU, CHEN, and DAUBERT[31] showed, that forms I and II correspond to β- and β′-forms respectively. Form III was not identified, but there is little doubt that this is the vitreous form, except for the case of C_{16}—C_{14}—C_{12}, where it is probably the α-form. This can be deduced by plotting the m.p.s of the related series, C_{14}—C_{12}—C_{10}, C_{16}—C_{14}—C_{12}, C_{18}—C_{16}—C_{14}, which have the same terminal

Table 12. Long and short spacings of triacid triglycerides[31], [41]

	Long		Short	
	β	β′	β	β′
C_{18}—C_{14}—C_{16} . .	63·4	41·5	3·83vs, 4·62vs, 5·24m	3·80vs, 4·11vs, 4·34vs
C_{18}—C_{12}—C_{16} . .	61·2	60·0	3·84vs, 4·33m, 4·61vs, 4·83m, 5·19m	
C_{18}—C_{10}—C_{16} . .	60·0		3·82vs, 4·21m, 4·61vs, 4·78m, 5·41m	
C_{16}—C_{14}—C_{14} . .	40·7		3·72vs, 3·82vs, 4·57vs, 5·34m	
C_{16}—C_{12}—C_{14} . .	59·8		3·83vs, 4·25s, 4·61vs, 4·8m, 5·37m	
C_{16}—C_{10}—C_{14} . .	57·3		3·82vs, 4·3m, 4·62s, 4·82m, 5·18s, 5·35m	
C_{16}—C_{18}—C_{12} . .	62·4	43·3	3·82vs, 4·34m, 4·62vs, 5·2m	3·82s, 4·11s, 4·29vs
C_{16}—C_{14}—C_{12} . .	59·6	40·1	3·82vs, 4·32s, 4·61vs, 4·82s, 5·02m, 5·42m	3·81vs, 4·26vs, 4·45m
C_{16}—C_{10}—C_{12} . .	54·9		3·82vs, 4·6vs, 5·28m	
C_{12}—C_{16}—C_{10} . .	38·5	59·0	3·85vs, 4·14vs, 4·33s, 4·82m, 5·35m, 5·55m	3·76s, 3·86s, 4·22vs, 5·14m
C_{12}—C_{14}—C_{10} . .	56·1	54·3	3·83vs, 4·2vs, 4·45s, 5·6s	3·73s, 3·84s, 4·05s, 4·19s, 4·45s, 4·58s
C_{12}—C_{12}—C_{10} . .	35·6	53·0	3·81s, 4·19vs	3·83s, 4·16vs, 4·29s, 4·46s
C_{16}—C_{14}—C_{12} . .	35·7		3·74s, 3·83s, 4·57vs, 5·08m, 5·33m	
C_{14}—C_{12}—C_{10} . .	33·4		3·82s, 4·13vs, 4·33vs, 4·6m	

irregularity, yet increase regularly in length (Fig. 15). Forms I and II lie on smooth curves, whereas the centre point of the lower curve is clearly too high.

From the absence of experimental details, this series does not appear to have

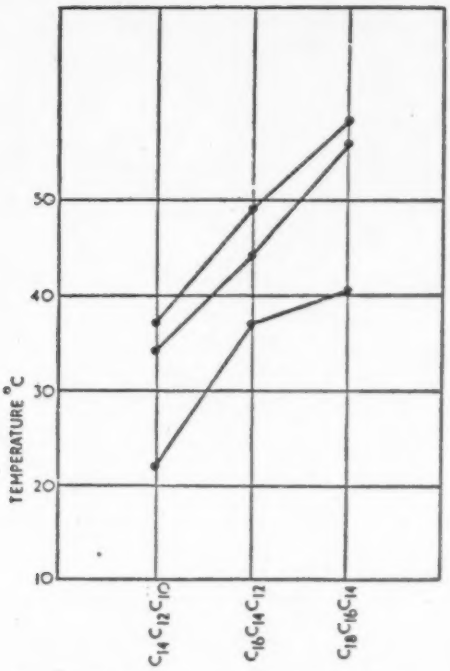


Fig. 15. Melting points of triacid triglycerides.

been submitted to a detailed thermal study; such a study would most probably reveal other α-m.p.s lying between those of forms II and III.

X-ray data for the above glycerides are given in Table 12.

Monoglycerides

1-*monoglycerides of saturated acids*

Fischer, Bergmann, and Bärwind[42] were the first to report the double melting of 1-mono-stearin and -palmitin in 1920. Ten years later, Rewadikar and Watson[43] made a systematic study of the melting phenomena of the even acid 1-mono-glycerides from monolaurin to monostearin, and showed that they exist in two distinct forms; they also suspected the existence of a third form, without, however, expressing a definite opinion. This work was confirmed and extended by Malkin and Shurbagy,[44] who, by means of cooling and heating curves and x-ray examination, found three solid modifications for all 1-mono glycerides from monodecoin to monostearin, viz. a low melting α-form (vertical rotating chains) and two higher melting forms, β′- and β- (inclined chains, alternating m.p.s). They showed that when the molten monoglyceride is cooled

the α-form is the first to separate; this then changes into a more stable β′-form, which finally changes much more slowly into the stable β-form. The latter can also be obtained by very slow crystallization from solvents; rapid crystallization from solvents may give rise to a mixture of all three forms, but as a rule gives the β′-form. LUTTON and JACKSON[45] later claimed to have found a fourth form which they termed sub-alpha.

Cooling and heating curves

The curves for monoundecoin in Fig. 16 illustrate the general behaviour of monoglycerides. AB, cooling to room temperatures, shows the separation of α-form, and BC shows the melting of α-, β′-, and β-forms. On increasing the

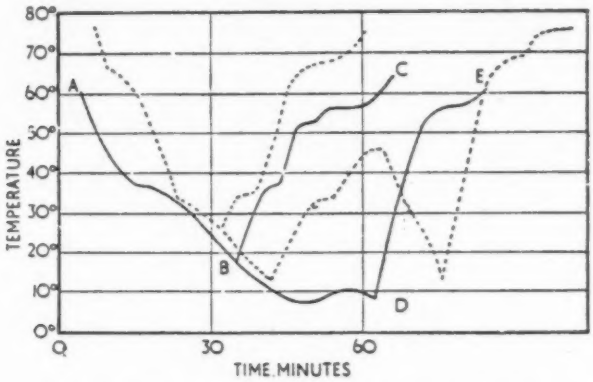


Fig. 16. Cooling and heating curves of 1-monoglycerides.
1-monoundecoin, ———; 1-monopalmitin, ·····.

cooling gradient by the use of ice, a lower arrest is observed (BD) and the subsequent heating curve, DE, now rises to the m.p. of the β-form. No arrest now appears at the α-m.p. and only the slightest indication of an arrest at the β′-m.p. Clearly, as the α-form cools, it changes into β′- and β-forms, and from BDE it appears that these transitions are completed by further cooling. The curves for monolaurin are very similar to the above, and curves for other members differ in a gradated manner, according to the length of the acid chain, changes being more rapid with shorter than with long chains. Thus the α and β′ arrests on the heating curve of monodecoin are less pronounced, because of the rapid $\alpha \rightarrow \beta'$, $\beta' \rightarrow \beta$ changes, whereas the corresponding arrests are more marked in the case of monotridecoin, and the final β-arrest is slight. On ascending the series to monomyristin and monopentadecoin, the β-arrest disappears altogether, owing to the slow $\beta' \rightarrow \beta$ change. If, however, the specimen is first cooled very slowly, then the latter transition takes place, and a β-arrest is observed on the heating curve. With still higher members, both the β′ and β arrests are usually absent; after very slow cooling, the β′-arrest may be observed but the transition $\beta' \rightarrow \beta$ can only be brought about by maintaining the specimen in the region of its β′-m.p. for several days.

The cooling curves for the series are alike in showing two arrests, the first of which represents the changes liquid → α-form. The changes at the second arrest are more complex, but for the lower members appear to be $\alpha \rightarrow \beta' \rightarrow \beta$-transitions. For the higher members, however, these transitions do not appear to take place, and the lower arrest is reversible (discontinuous curve, Fig. 16, 1-monopalmitin).

Table 13

1-*Monoglyceride*	α	β′	β	*Lower arrest*
Decoin . .	27°	49°	53°	8°
Undecoin . .	36·5	52	56·5	3
Laurin . .	44	59·5	63	15
Tridecoin . .	50	61	65	9
Myristin . .	56	67·5	70·5	24
Pentadecoin . .	62	69	72	17
Palmitin . .	66·5	74	77	34
Heptadecoin .	70	74·5	77	28
Stearin. . .	74	79	81·5	47·5 and 42*

* 1-Monostearin differs from the other monoglycerides in that there are two distinct reversible changes in this region. LUTTON[380] has now confirmed this, having previously failed to observe the two changes.[443]

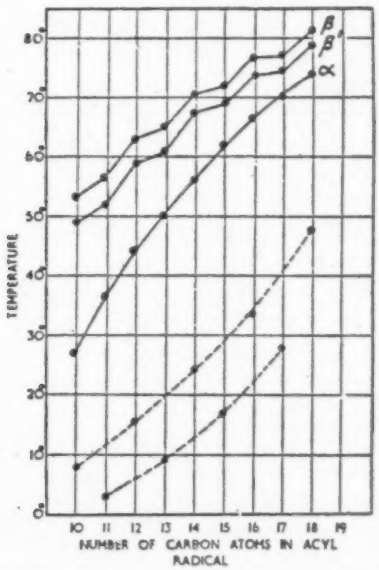


Fig. 17. Melting points and transition temperatures of 1-monoglycerides.

The various curves can be modified by varying the rate of cooling. Slower cooling increases the velocity of the transitions, so that if, for example, monopalmitin is cooled very slowly, it gives the type of curve normally given by trimyristin, and shows evidence of the β′-form on the heating curve. Conversely

the curves for monomyristin cooled rapidly are similar to those shown in Fig. 16 for monopalmitin, and the lower arrest appears to be reversible. This reversible effect is therefore due to the excessively slow rate of the transition $\alpha \rightarrow \beta'$, and if the palmitin curve is continued as shown in Fig. 16, F.G.H., the magnitude of the reversible effect diminishes, and the presence of the β'-form is shown by the final heating curve.

Melting points of the various forms and temperatures of the lower arrests on the cooling curves are given in Table 13 and plotted in Fig. 17.

X-ray investigation

The spacings of the stable β-forms are best obtained from specimens which have been slowly crystallized from hexane or ether. Rapid crystallization gives rise to mixtures of forms, mainly the β', mixed with the β-form for the lower members of the series, and the α-form for the higher. Melted layers give rise to α-, β'-, or β- forms, according to the rate of cooling and the temperature during the X-ray exposure. Thus, a specimen allowed to solidify on a temperature controlled mount, just below its α-m.p., gives the α-spacing, together with that of the β' or β-form, transition into which is favoured by the high temperature.

Within experimental error, the long spacings of β'- and β-forms are the same; they correspond to double molecules tilted at an angle of $54\frac{1}{2}°$. The long spacings of α-forms correspond to double molecules lying vertically across the reflecting planes.

Table 14. Long spacings of saturated 1-monoglycerides[44]

No. of C atoms in acid	10	11	12	13	14	15	16	17	18	20*
β' and β-forms	32·9	35·2	37·3	39·6	41·5	43·8	45·8	48·2	50·0	54·4
α-form	37·2	40·2	43·2	46·2		51·3			58·3	

Short spacings of saturated 1-monoglycerides

$\beta' = 3{\cdot}86$m, $4{\cdot}24$s: $\beta = 3{\cdot}88$s, $4{\cdot}12$, $4{\cdot}37$, $4{\cdot}58$s.

* β-form determined by SIDHU and DAUBERT.[44]

The α-form is stable only over a small temperature range near its m.p. and at this temperature it rapidly changes into the more stable β'-form. It is rare to obtain more than two orders on the X-ray photograph, and as a rule only the first order appears, accompanied by several orders of the β'-form.

Although the long spacings of the β'- and β-forms are the same, the two forms are readily distinguished by their short spacings, the strongest lines of which are 3·86m 4·24s, and 3·88s 4·58s, respectively. The increasing rate of transition $\beta' \rightarrow \beta$ with decreasing length of acid chain is well shown by the short spacings. From C_{15} upwards, β'-forms appear to be indefinitely stable at room temperature, with monomyristin the change takes place overnight and with lower members the transition usually occurs during the time of an X-ray exposure.

The α-form may occasionally give rise to a single strong line at 4·18 Å, but as

a rule this is accompanied by weaker lines on either side, which suggests that transitions are taking place during the exposure. LUTTON and JACKSON[45] state that the α-form is reasonably stable down to the lower transition temperature, but it is clear from their X-ray results that they were not dealing with the somewhat fleeting α-form, e.g. the long spacing for their alleged α-form of monostearin is 8 Å too short, and it cannot therefore be the spacing of a rotating vertical form.

The instability of the α-form is well demonstrated by microscopic examination. If a monoglyceride is melted on a microscope slide under a coverslip, and placed on a heated stage just below the m.p. of the α-form, it appears through crossed nicols as a greyish liquid, without structure; yet nevertheless, it gives a strong uniaxial interference figure. This condition usually lasts only a few minutes, and

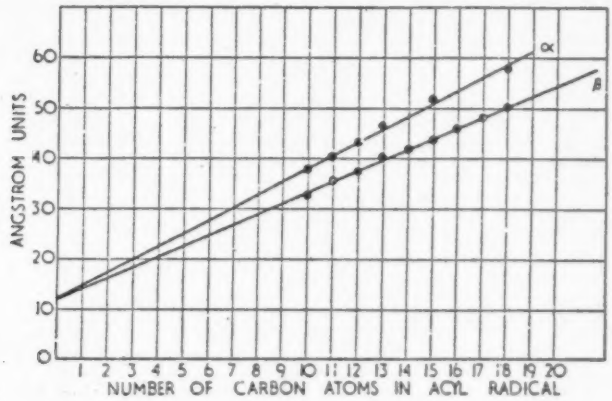


Fig. 18. Long spacings of saturated 1-monoglycerides.

considerable repetition is necessary in order to observe it. This is the true α-form, with vertical rotating chains, such as has been observed for fatty acid esters[46] and alcohols.[47], [48] Although the α-forms of triglycerides give rise to hexagonal spacings similar to those of α-forms of monoglycerides, they do not exist in a form which gives a single uniaxial interference figure, and hence their chains are to be regarded as non-rotating, but frozen into positions which simulate the structure of true α-forms.

Under the microscope, the liquid-like α-form of monoglycerides rapidly passes into a variety of irregular shapes, typical of the mesomorphic forms associated with liquid crystals, and MALKIN and SHURBAGY considered that this form changed on cooling into a semi-vitreous solid, slightly more ordered than the vitreous form of triglycerides. LUTTON and JACKSON consider that this latter solid is a new crystalline modification, termed sub-alpha, which changes reversibly on heating into the α-form. They find that it gives a distinctive group of short spacings (4·13vs, 3·92m, 3·75m, 3·58m), and there is no doubt that these spacings can be obtained for myristin upwards, when the molten glyceride is solidified fairly quickly. The cooling and heating curves already discussed, particularly those for monopalmitin, show, however, that the α → sub-alpha

change is not genuinely reversible, but only appears to be so because of the slow rate of the $\alpha \rightarrow \beta'$ transition; cf. for example, the interesting dielectric constant study by CROWE and SMYTH,[49] where it is shown that 1-monopalmitin and stearin take several hours to pass from the α- to the β'-form in the neighbourhood of the α-m.p. Whatever the true nature of this sub-alpha form, it is mainly non-crystalline in the ordinary sense of the term, for taking similar acid chains, the monoglycerides crystallize much more slowly than triglycerides, and are thus more likely to solidify in a vitreous phase on being rapidly cooled.

This slow crystallization is probably due to the possibility of a variety of modes of hydrogen bonding between the two free hydroxyl groups of each molecule, which make it more difficult for the molecules to assume the correct position for crystallization, e.g. the slow crystallization of polyhydroxy compounds such as glycerol and sugar is well known. Moreover, the high m.p. of 1-monoglycerides, compared with alcohols and acids, suggests that the structure is more complex, and may involve lateral hydrogen bonding in addition to the normal head to head bonding. The still higher melting acid amides afford a parallel example. It is of interest to note that the 2-monoglycerides, which on grounds of symmetry might have been expected to melt higher than the 1-isomers, actually melt several degrees lower. This suggests that the hydrogen bonding of the former is of the head to head type.

2-Monoglycerides

The only thermal investigation of 2-monoglycerides is due to DAUBERT and CLARKE,[51] who examined a series from monocaprin to monostearin, and who came to the conclusion that they were not polymorphic. FILER Jr., SIDHU, DAUBERT, and LONGENECKER[21] had earlier determined the long and short spacings of the solvent crystallized forms of this series, and although their data are complicated by an omission to filter β-radiation adequately, their results are in excellent agreement with the unpublished data of BEVAN and MALKIN given in Table 15.

Table 15

	M.P.	*S.P.*	*Long spacing**	*Short spacings*
2-Monocaprin . .	40·2	34·0	29·4	4·07, 4·38, 4·58
2-Monolaurin . .	51·0	47·5	32·8	3·96, 4·40, 4·58
2-Monomyristin . .	61·3	58·0	36·2	3·92, 4·42, 4·60
2-Monopalmitin . .	69·0	65·5	40·4	3·88, 4·42, 4·60
2-Monostearin . .	75·2	70·0	43·9	3·86, 4·43, 4·60

* These correspond to double molecules tilted at an angle of 45°30′. When plotted against the carbon content of the acids, the intercept at $C = 0$ is $\simeq 11$Å.

A close examination of cooling curves shows, however, that 2-monoglycerides probably separate from the melt in the α-form. For mono-caprin to monomyristin, this changes rapidly into the β-form, and the only arrest on the heating curve is at the β-m.p. Cooling curves for monopalmitin and monostearin show a

second arrest at a lower temperature. This appears to represent a reversible change, for there is an arrest at the same temperature on the heating curve, which then rises to the β-m.p. There is thus a certain parallel with the behaviour of the β'-monoglycerides, but the transitions are much more rapid, and there is no indication of the existence of the β'-form. Curves for 2-monostearin and 2-monomyristin are given in Fig. 19.

All the 2-monoglycerides exhibit spherulite formation if cooled at a suitable rate, and with a little care, 2-monocaprin may be shown microscopically, to exist in a uniaxial form.

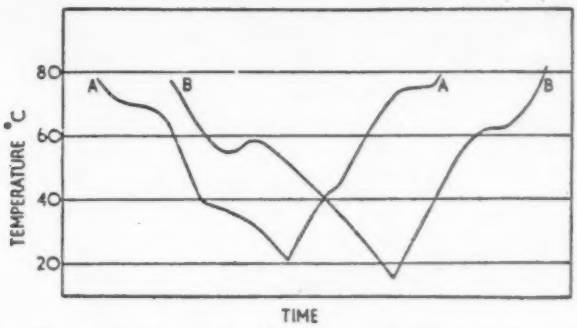


Fig. 19. Cooling and heating curves of 2-monoglycerides. 2-monostearin (AA); 2-monomyristin (BB).

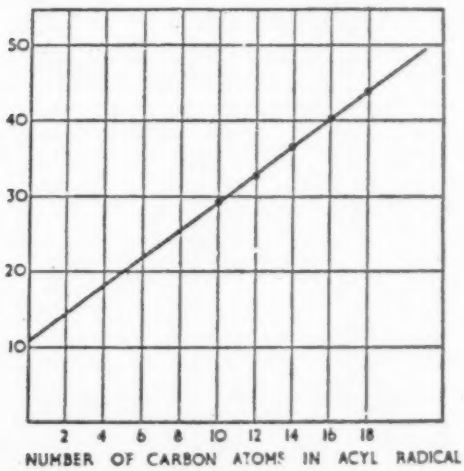


Fig. 20. Long spacings of 2-monoglycerides.

1,3-*Diglycerides*

The polymorphism of simple diglycerides was first investigated by MALKIN, SHURBAGY, and MEARA,[52] who showed that they were similar to the mono- and triglycerides in existing in α-, β'-, and β-forms. They reported an apparent discontinuity at dipentadecoin, since only one β-form was obtained for the higher members, but later, BAUR, JACKSON, KOLP, and LUTTON[53] observed the missing β-form for dipalmitin and distearin: hence, the entire series from dicaprin to

distearin, behaves normally, and exists in α-, β′-, and β-forms. The latter authors showed that diglycerides often crystallize from solvents in the β′-forms, and they also observed a new type of short spacing, very similar to those described by MALKIN *et al.*, but as there were now three types of short spacings to describe two forms (β′ and β), there remained some slight confusion, which was removed by a later re-examination of the problem by HOWE and MALKIN.[54]

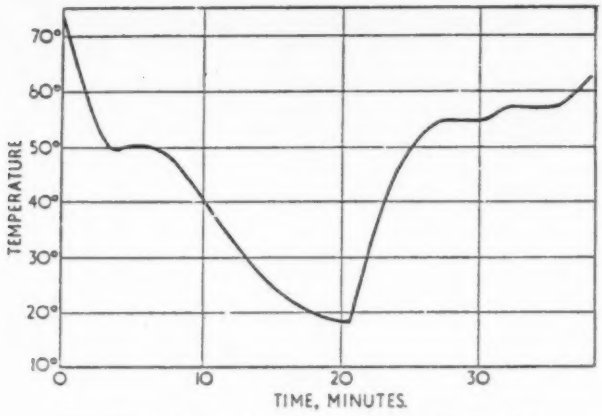


Fig. 21. Cooling and heating curves for 1,3-dilaurin.

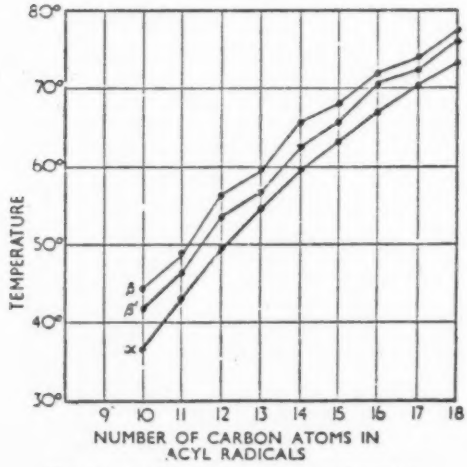


Fig. 22. Melting points of 1,3-diglycerides.

SIDHU and DAUBERT[55] have shown that a number of mixed diglycerides exhibit the same type of polymorphism as the simple diglycerides.

Diglycerides give rise to spherulites which are distinguished from those given by triglycerides by a curious rippled effect.

Cooling and heating curves

Cooling curves show only one arrest (α-separation), but two usually appear on the heating curves. For the lower members, with not too rapid cooling, these

are at the β'- and β-m.p.s, whilst for the higher members, or for the lower members after rapid cooling, they occur at the α- and β'-m.p.s The curves shown in Fig. 21 for dilaurin are typical of those for the lower members. In general, $\alpha \rightarrow \beta'$ transitions are rapid, but $\beta' \rightarrow \beta$ transitions are unusually slow and for the higher members, occur only near the β'-m.p. BAUR *et al.* have shown that the latter change takes place at rates which are inversely related to the chain length.

Melting points are given in Table 16, and those of the series dicaprin to distearin are plotted in Fig. 22.

X-ray investigation

X-ray data for β'- and β-forms only have been recorded. Owing to the rapidity of the transition $\alpha \rightarrow \beta'$, it has not been found possible to obtain X-ray data for

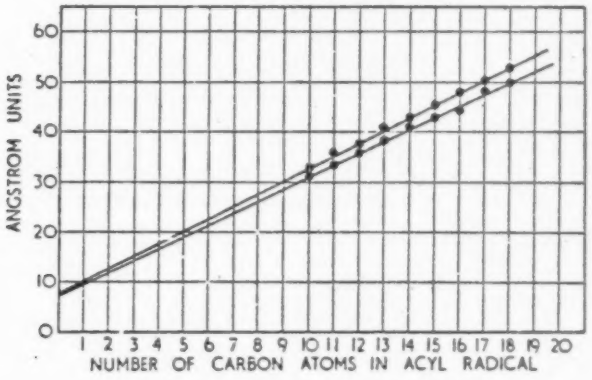


Fig. 23. Long spacings of 1,3-diglycerides.

the α-form, and the identity of the low melting form as an α-form, can only be inferred by analogy with the behaviour of other glycerides, and by the fact that the m.p.s are non-alternating (see Fig. 22).

The long spacings of the simple diglycerides correspond with double molecules tilted at angles of $75\frac{1}{2}$ (β) and $64\frac{1}{2}$ (β'). When plotted against carbon atoms in the acyl chain, the intercepts are $\simeq$ 8A, see Fig. 23. It is seen from Table 16 that there are three distinct groups of short spacings, which have been termed the a, b, and c types. They occur as follows:

a type: β-forms of all odd acid 1,3-diglycerides, and β'-forms of dipalmitin and distearin.
b type: β'-forms of all 1,3-diglycerides except dipalmitin and distearin.
c type: β-forms of all even acid diglycerides.

These results emphasize the difficulty of attempting to classify polymorphic forms of glycerides according to the type of short spacing (see p. 13). Both β'- and β-forms of diglycerides give rise to the strong 4·6 line, which according to LUTTON's classification, is characteristic of β-forms only.

Table 16. Melting points and x-ray data for 1,3-diglycerides[52], [54]

	Melting points			*Long spacings A*		*Short spacings, A**	
	α	β′	β	β′	β	β′	β
Didecoin	37°	42°	44·5°	30·5	32·5	b 4·65s, 3·03m, 3·7s, 3·46w	c 4·55 3·75
Diundecoin	43·5	47	49	33·2	35·2	b 4·63s, 3·88w, 3·72s, 3·58w	a 4·53 3·81 3·66
Dilaurin	49·5	54	56·5	35·7	37·4	b 4·66s, 3·94m, 3·73s, 3·61vw	c 4·6 3·75
Ditridecoin	54·5	57	59·5	38·1	40·4	b 4·58s, 3·9w, 3·71s, 3·53w	a 4·55 3·83 3·68
Dimyristin	60	63	65·5	40·5	42·6	b 4·64s, 3·88m, 3·68s, 3·53vw	c 4·57 3·73
Dipentadecoin	63·5	66·5	68·5	42·5	45·0	b 4·58s, 3·86w, 3·70s, 3·52w	a 4·58 3·84 3·68
Dipalmitin	68	71·5	72·5	44·7	47·5	a 4·6s, 3·87s, 3·65s	c 4·6 3·75
Diheptadecoin	71·5	72·5	74·5	47·7	50·3	b 4·6s, 3·85m., 3·7s, 3·54w	a 4·57 3·82 3·66
Distearin	74	77	78	49·5	52·8	a 4·6s, 3·88s, 3·68s	c 4·6 3·73
1-Myristyl-3-caprin*	30	44	48	—	38·0	—	c 4·55 3·84 3·73
1-Palmityl-3-laurin*	51	56	59·5	—	42·7	—	c 4·03 3·8 3·70
1-Stearyl-3-myristin*	56	63	67	—	47·6	—	a 4·6 3·70

* SIDHU and DAUBERT,[61]

1,2-*Diglycerides*

The only study of the polymorphism of 1,2-diglycerides is due to Howe and Malkin,[54] who showed that they exist in two solid modifications, α and β, the transition α → β being much slower than is the case with the 1,3-isomers.

Thermal examination

Solvent crystallized material melts at the β-m.p. and resolidifies at the α-m.p., remelting again at the α-m.p. The transition α → β is very slow, but if α-forms are maintained overnight, just below their m.p.s they change into the β-forms. Cooling and heating curves normally show arrests at the α-m.p. only.

1,2-Diglycerides exhibit spherulite formation, but the rippled appearance characteristic of spherulites of 1,3-diglycerides is absent.

X-ray examination

Long spacings of α- and β-forms are obtained from "melted" and pressed layers respectively and short spacings from "melted" and pressed rods. When plotted against the carbon content of the acyl chain, they fall on two straight lines which cut the axis C=O, at ≏ 6·5 (β) and 9·5 Å (α). The results agree with an arrangement of double molecules lying vertically (α) or inclined at an angle of ≏ 64° (β) to the reflecting planes. Short spacings are practically identical for the series, and they differ in type from those given by the 1,3-diglycerides. M.p. and x-ray data are given in Table 17.

Table 17. Melting points and x-ray data for 1,2-diglycerides[54]

	α-Form			β-Form				
	M.P.	*Long spacing, Å*	*Short spacing, Å*	*M.P.*	*Long spacing, Å*	*Short spacing, Å*		
						m	vs	s
Dilaurin	20·0°	39·2	4·13	39°	34·1	4·31	4·01	3·79
Dimyristin	37·5	44·4	4·12	54	38·8	4·31	4·06	3·82
Dipalmitin	50·0	49·3	4·10	63·5	43·5	4·27	4·03	3·76
Distearin	59·5	54·5	4·12	71	48·3	4·27	4·05	3·81

Unsaturated glycerides

Glycerides containing unsaturated acids exhibit the same type of polymorphism as the saturated glycerides, and there are no major differences. Indeed, those containing *trans* unsaturated acids are very closely similar in behaviour and structure to glycerides of the corresponding saturated acids. As might be expected, however, from the slight irregularity which a *cis* bond introduces into the chain, there are structural differences in the case of *cis*-acid compounds. This is particularly noticeable with oleo-disaturated triglycerides, which have a general tendency to crystallize in triple-chain-length structures, irrespective of the relative lengths of the chains.

Table 18

	Vitreous	α	β′	β	*Long spacings*			*Short spacings*	
					α	β′	β	β′	β
Triolein	− 32*	−12*	—	4·9	45·2	45·8	43·3	4·35, 3·87	5·28, 4·57s, 3·97, 3·84, 3·71
Trielaidin	15·5	37	—	42	—	—	44·1		5·3, 4·6s, 3·9, 3·7
Trierucin	6	17	25	30	—	54·7	51·1	4·53, 4·28, 3·88	5·24, 4·6s, 4·03, 3·84, 3·7
Tribrassidin	43	50	—	59	59·3	—	53·6		5·3, 4·6s, 3·9, 3·7
	Form I							*Form II*	
Trilinolein:									
Wheeler *et al.*		− 13·1				—		− 43	
Daubert *et al.*		− 12·9				—		− 45·6	
Ravich *et al.*		− 10·5(β)				− 27–29°(α)		− 43–47°(vitreous)	
Trilinolenin		− 24·2						− 44·6	

* Ferguson and Lutton term these α and β′, but it is unlikely that there is so great a difference in melting point between β′- and β-forms (see p. 22).

Simple triglycerides

The polymorphism of simple unsaturated triglycerides has been investigated by CARTER and MALKIN,[56] who found three forms for trielaidin and tribrassidin, and four forms for trierucin. WHEELER, RIEMENSCHNEIDER, and SANDO[57] reported three forms for triolein and two for trilinolein, and the latter result was confirmed later by DAUBERT and BALDWIN,[58] who also found two forms for trilinolenin. RAVICH and ZURINOV,[59] however, have since reported three forms for trilinolein. The above investigations were carried out by means of m.p.s and cooling and heating curves, and only that of CARTER *et al.* included X-ray results. However, in 1947, FERGUSON and LUTTON[60] succeeded in obtaining characteristic X-ray data for the various forms of triolein. They confirmed the m.p.s reported by WHEELER *et al.*, and brought out similarities in X-ray data with the results of CARTER *et al.* for trierucin. X-ray and m.p. data are given in Table 18.

In general, transitions with the above glycerides are more rapid than for the corresponding saturated compounds, hence the difficulty in completing the above table. It will be noticed that the short spacings of the β-form of triolein and trierucin, are identical within experimental error.

Unsaturated mixed triglycerides

DAUBERT and CLARKE[61] showed by means of cooling and heating curves that 2-oleo-disaturated glycerides of capric, lauric, myristic, palmitic, and stearic acids, exist in four solid modifications, and this was supported by the work of MEARA,[62] who found four m.p.s for 2-oleodistearin, isolated from various natural fats. Later, FILER Jr., SIDHU, DAUBERT, and LONGENECKER[21] recorded X-ray data for certain of the above glycerides, and shortly afterwards LUTTON[65] examined 2-oleodistearin thermally and with X-rays, and concluded that only three forms exist. In addition to the difference concerning the number of polymorphs, LUTTON's X-ray results were not in agreement with those of FILER *et al.*, and this led MALKIN and WILSON to re-examine 2-oleo-dimyristin, -dipalmitin, and -distearin. They were able to report excellent agreement with the thermal investigations of DAUBERT and CLARKE, whose curves they were able closely to reproduce. They also found a further modification, and showed that these compounds exist in the following five modifications: vitreous, α, β'', β', β, in order of ascending m.p.

2-Elaido-dipalmitin and -distearin, which were examined at the same time, were found to resemble the related 2-stearodipalmitin and tristearin respectively, and to exist in four forms, viz. vitreous, α, β', and β.

Cooling and heating curves

The curves of DAUBERT and CLARKE for 2-oleodimyristin reproduced in Figs. 24 and 25 are typical for the series. They are the normal curves for low melting glycerides, showing separation of either the α (AA') or the vitreous form (CC') according to the rate of cooling, followed by transitions into other forms, according to the time interval before the heating curve is taken. The series is very similar to the symmetrical mixed triglycerides of the 2-caprodilaurin

series, which is characterized by rapid transitions vitreous → α → β′, e.g. cf. Figs. 3 and 25 (very slow cooling). The interpretation of these curves will be clear from the earlier discussion on page 3ff. The curves given in Fig. 24

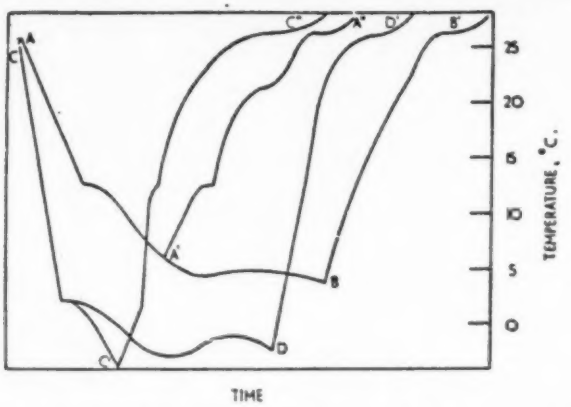
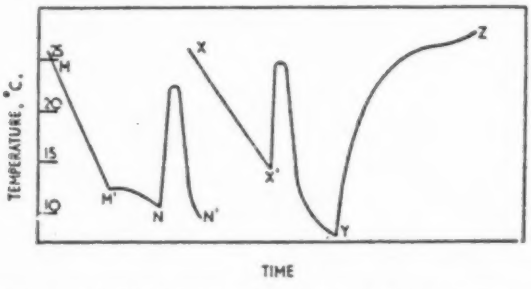


Fig. 24. Cooling and heating curves for 2-oleodimyristin (DAUBERT and CLARKE).



Figs. 25. Cooling and heating curves for 2-oleodimyristin (DAUBERT and CLARKE).

definitely establish the existence of four forms, yet the upper m.p. (26·5) is still two degrees lower than the m.p. of the solvent crystallized material. This led MALKIN and WILSON to the discovery of the fifth form, the stable

Table 19. Melting points of 2-oleo- and 2-elaido-disaturated glycerides[64]

	Vitreous	α	β″	β′	β
2-Oleodimyristin .	2·0 (2·1)	11·0(12·3)	19·0(21·5)	26·5(26·3)	28·5
2-Oleodipalmitin .	12·0(12·0)	21·5(20·8)	29·0(30·4)	35·0(35·2)	37·5
2-Oleodistearin . .	23·0(22·3)	29·5(29·8)	37·0(37·0) (37·6)	41·5(41·6)	43·5
2-Elaidodipalmitin .	33·0	42·0	—	52·5	55·0
2-Elaidodistearin .	40·0	46·0	—	58·0	61·0
	IV	III	II	I	
2-Oleodicaprin . .	(— 16·4)	(— 10·2)	(0·6)	(6·2)	
2-Oleodilaurin . .	(— 7·3)	(1·4)	(11·0)	(16·5)	

Values in parentheses are due to DAUBERT and CLARKE.[41]

Table 20. X-ray data for 2-oleo- and 2-elaido-disaturated glycerides[64]

	Form	Long spacing, A	Short spacings, A
2-Oleodimyristin*	β'	39·1	3·92s, 4·23s
2-Oleodimyristin*	β	56·7	3·68m, 3·84m, 4·04m, 4·58vs, 5·17w, 5·43m
2-Oleodipalmitin†	β''	68·5	3·58m, 3·87vs, 4·42m, 4·74s, 5·21w
2-Oleodipalmitin†	β'	42·1	3·88m, 4·14m, 4·35s
2-Oleodipalmitin†	β	60·7	3·7m, 3·8w, 4·0m, 4·56vs, 5·45m
2-Oleodistearin	α	50·3	4·17s
2-Oleodistearin	β''	72·5	3·58m, 3·88vs, 4·47m, 4·74s, 5·22w
2-Oleodistearin	β'	45·2	3·85m, 4·11m, 4·32s
2-Oleodistearin	β	64·8	3·66m, 3·84m, 4·02m, 4·58vs, 5·44m
2-Elaidodipalmitin†	β'	44·7	3·8m, 4·18s
2-Elaidodipalmitin†	β	42·7	3·77s, 4·04w, 4·17s, 4·34m
2-Elaidodistearin	α	51·2	4·14s
2-Elaidodistearin	β'	47·2	3·8m, 4·17s
2-Elaidodistearin	β	44·9	3·66m, 3·85m, 4·00w, 4·57s, 5·31m

s = strong; vs = very strong; m = moderate; w = weak.
* α- and β''-forms unstable at room temperature.
† α-form unstable at room temperature.

β-form, which is normally only obtained from solvents, since the change $\beta' \rightarrow \beta$ is exceedingly slow. This is true also for other members of this group, and for 2-oleodistearin, the change $\beta'' \rightarrow \beta'$ is also slow, and only takes place when the β''-form is held near its m.p. for a few hours. A heating curve for this compound, therefore, shows only one arrest—at the β'' m.p. M.p. and X-ray data are given in Tables 19 and 20.

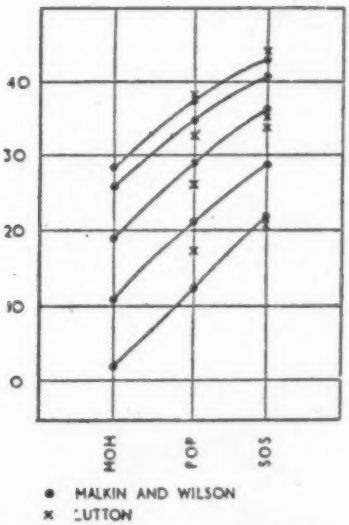


Fig. 26. Melting points of 2-oleo-disaturated glycerides.

In a later paper dealing with 2-oleo-dipalmitin and -distearin, LUTTON and JACKSON[65] failed to observe the forms reported by MALKIN and WILSON and recorded widely divergent m.p. and X-ray data. Since, however, their m.p.

data for 2-oleodistearin are greatly different from those of DAUBERT and CLARKE, MALKIN and WILSON, and MEARA, which are in agreement, and further, since they show no relationship to those of the related oleodipalmitin (see Fig. 26), some doubts may be expressed concerning the purity of their compounds.

Similar doubts arise concerning the results reported in an x-ray study for further oleo-disaturated glycerides, by LUTTON,[66] m.p. data for which are given in Table 21.

Table 21. 2-Oleo-disaturated glycerides

POP		*POS*		*SOS*	
Alpha-2	18·1	Alpha-2	18·2	Alpha-3	22·4
Sub-beta prime-2	26·5	Beta prime-2	?	Beta prime-3	35
Beta prime-2	33·5	Beta prime-3	33	Sub-beta-3	36·2
Beta-3	38·3	Beta-3	38	Beta-3	44·3

1-Olec-disaturated glycerides

OPP		*OPS*		*OSP*		*OSS*	
Alpha-3	18·5	Alpha-2	25·3	Sub-alpha-2	26·3	Alpha-3	30·4
Beta prime-3	35·2	Sub-beta-prime-3	37	Beta prime-2	40·2	Beta prime-3	43·5
		Beta prime-3	40	Beta prime-3	39·8		

It is not easy to see why the m.p.s of the α-forms of the isomeric SOS and OSS differ by so much, whereas those of the related isomers POP and OPP are the same, nor is it clear why the α-m.p.s of POS, OPS, and OSP also differ

Table 22

	I	*II*	*III*	*IV*
SSO	38·5	—	—	26·7
PPO	34·5	—	29·8	18·5
MMO	25·0	22·7	18·6	3·8
LLO	16·0	4·8	− 10·0	− 15·5
CCO	4·0	− 2·5	− 15·0	− 27·0
SOO	23·5	—	8·6	− 1·5
POO	19·0	—	2·5	− 13·2
MOO	13·5	—	− 4·2	− 21·8
LOO	6·5	—	− 10·9	− 22·0
COO	0·5	—	− 16·5	− 40·5
CyOO	− 5·6	—	—	− 50·0
CoOO	− 10	—	− 34·2	− 56·5

Co = caproic; Cy = caprylic; C = capric; L = laurie, etc.

greatly. As a rule, isomerism of this type results in a difference of only 2 or 3°. It is also extremely unlikely that any member of this group exists in only two forms, and it is clear that this group should be submitted to a thorough investigation of cooling and heating curves before x-ray data are allocated to the various forms.

T.p. data for a number of triglycerides containing the oleic radical, determined by DAUBERT and CLARKE[67] are given in Table 22. These authors did not carry out any x-ray investigation, and therefore denoted the different forms by I, II, III, and IV.

1-*Mono- and* 1,3-*di-glycerides*

1-*Monoglycerides*—The upper and lower curves in Fig. 16, p. 30, are practically identical in form with those of 1-monobrassidin and 1-monoerucin, respectively. In the case of the latter, the transitions vitreous $\rightarrow \alpha$, $\alpha \rightarrow \beta'$, $\beta' \rightarrow \beta$ take place at room temperature, and according to the duration of the cooling curve, arrests for α- and β'-forms may or may not appear on the heating curves. The slowest transition is the $\beta' \rightarrow \beta$ and an arrest at the β-m.p. is not observed unless the curves are taken slowly.

1-Monoolein can occasionally be obtained in a form which gives a uniaxial interference figure.

1,3-*Diglycerides*—Cooling and heating curves are similar to those given in Fig. 21 for dilaurin except that the arrest at the β-m.p. is slight, or more usually, absent. The transition $\beta' \rightarrow \beta$ is particularly slow and β-forms are best obtained by very slow crystallization from solvents.

x-ray and m.p. data are given in Table 23.

Glycerides of chaulmoogric and hydnocarpic acids

GUPTA and MALKIN[71] have recently examined the 1-mono, 1,3-di-, and triglycerides of the above acids and find that they exhibit the same type of polymorphism as the glycerides of straight chain fatty acids. Their results are summarized in Table 24.

1-*Monoglycerides*

The first form to separate from the melt is the α-form which changes slowly, at room temperature, into the β'-form. When the experiment starts with the molten glyceride, these are the only forms observed. The high-melting β-form is obtained by slow crystallization from solvents, and it is not easy to obtain it entirely free from β'-form. In contrast to the monoglycerides of straight-chain fatty acids, whose long spacings for β'- and β-forms are identical, the long spacings of the two forms are quite distinct, and correspond to tilts of 31° for the β-form and 44° for the β'-form. α-Forms, before changing to the β-form, pass quickly into an intermediate phase, the short spacings of which are the same as those given by saturated 1-monoglycerides, i.e. a strong line at 4·2 Å, associated with a few weaker lines close on either side.

Table 23

	Melting points				Long spacings		Short spacings											
							β′					β						
	Vitreous	α	β′	β	β′	β												
1-Monoolein	12·5	25	32	35		49·5						3·58,	3·77,	4·05,	4·32ms,	4·58s		
1-Monoelaidin	29·5	42	56	58·5		50·3	3·59,	3·73,	3·88,	4·13s		3·93s,	4·14,	4·38,	4·55s			
1-Monoerucin	15	36	47	50		58·3	3·68,	3·87,	4·05,	4·3,	4·65s	3·61,	3·75,	3·9,	4·08,	4·33m,	4·57s	
1-Monobrassidin	37	62	68·5	71		58·8	3·59,	3·75,	3·9,	4·14s		3·93s,	4·15,	4·4,	4·56s			
1,3-Diolein	—	18	—	25		30·7						3·64,	3·85ms,	4·06m,	4·23,	4·44m,	4·7s	
1,3-Dielaidin	—	49	53	55	49·8	52·6	3·6,	3·75s,	3·95,	4·61s		3·7s,	3·9s,	4·6s				
1,3-Dierucin	—	41	44·5	46·5	47·8	46·4	3·66m,	3·85m,	4·04,	4·24,	4·68s	3·64m,	3·95ms,	4·05m,	4·28,	4·38,	4·7s	
1,3-Dibrassidin	—	63·5	66·5	68·5	58·8	63·1	3·57,	3·75s,	4·0,	3·59s		3·72s,	3·91s,	4·6s				
1-Stearyl 3-olein*	—	—	—	52	30·0	48·2	3·88s,	4·67vs				3·78vs,	4·62vs,	4·74s				
1-Stearyl 3-elaidin*	—	—	62	65·9	50·0	52·6	3·75vs,	3·9vs,	4·6vs			3·74vs,	3·86vs,	4·58vs				
1-Oleyl 3-elaidin*	—	—	—	28·5	—	48·4						3·70s,	3·81s,	4·65s				
1,3-Diolein†				21·5		39·3						3·82,	4·73					
1,3-Dilinolein†				— 2·6		45·2						4·2,	4·69					
1,3-Dilinolenin†				— 12·3		40·3						4·4,	4·7					

* Benedict, Sidhu and Daubert,[66] see also Daubert and Sidhu[65] for data for 1,3-dielaidin.
† Daubert and Lutton.[19]
Other data from Carter and Malkin.[64]

Table 24. Melting points and x-ray data for glycerides of chaulmoogric and hydnocarpic acids

	Melting points				*Long spacings (Å)*		*Short spacings (Å)*	
	Vitreous	α	β′	β	β′	β	β′	β
1-Monochaulmoogrin . .		53·5°	57·5°	58·5–59°	43·5	38·1	3·8w, 3·98s, 4·58vs	3·9s, 4·1s
1-Monohydnocarpin . .		39·5	47	49	40·0	35·4	3·8w, 4·0s, 4·6vs	3·9s, 4·1s
1 : 3-Dichaulmoogrin . .		52	57	59	—	36·7	—	3·1w, 3·9m, 4·2m, 4·6s
1 : 3-Dihydnocarpin . .		42	47	49	—	32·7		3·4w, 3·7m, 4·0m, 4·6s
Trichaulmoogrin . . .	27°	35	41·5	44·5	39·2	39·2	4·0m, 4·3m, 4·6s	4·1s, 4·6s
Trihydnocarpin . . .	15	24	31	34	36·1	36·0	4·05m, 4·32m, 4·6s	4·1s, 4·58s

1,3-Diglycerides

These differ from most other diglycerides in crystallizing in large flakes, very similar in appearance to straight-chain fatty acids. Under the microscope, between crossed nicols, they exhibit a striking spherulite formation. Three distinct forms can be detected by the capillary melting-point methods, viz. α, β', and β, but the changes $\alpha \rightarrow \beta' \rightarrow \beta$ are so rapid that only the α- and the β-forms are observed on the cooling and heating curves. When the molten glyceride is cooled in a capillary, there is solidification at the α-m.p. and, if the temperature is raised when only a small portion has solidified, remelting occurs at the same temperature. It is not easy, however, to avoid transition to the β'- and even to the β-form, and some repetition is usually necessary to observe the m.p.s of the α- and the β'-forms. X-ray data could be determined only for the stable β-form, and the allocation of the α-structure to the lowest-melting form is based on analogy. From the long spacings, the tilt of the chains is found to be $\doteq 50°$.

Triglycerides

Starting with the molten glycerides, both capillary m.p.s and cooling and heating curves show the existence of vitreous, α- and β'-forms. The β-form can be obtained only by slow crystallization from non-polar solvents, or by holding the β'-form near its m.p. for some hours. Crystallization from ethanol or acetone usually gives the β'-form. Cooling curves (cooling jacket from 0° to room temperature) fall to the vitreous m.p. and then rise to the α-m.p. and, if the heating curve is taken when the curve again begins to fall, there is a single arrest at the β'-m.p. Thus, the change $\alpha \rightarrow \beta'$ is moderately rapid. Within experimental error, the long spacings of the β'- and the β-forms are identical, but the two forms are distinguished by their short spacings. Both triglycerides exhibit typical spherulite formation.

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2

AUTOXIDATION OF FATS AND RELATED SUBSTANCES

Ralph T. Holman

Introduction

Since antiquity, rancidity has been recognized as a problem in the storage of fats. Prolonged exposure of edible fats and oils to air, heat, and light eventually results in the development of unpleasant odours and flavours, rendering the fatty substance unpalatable and even toxic. The protection of foods against rancidity has remained an art until recent times, when systematic efforts have been made to discover the nature and causes of rancidity.

Chemical changes of several types contribute to what is known by the generic term rancidity. In its broadest meaning, rancidity denotes a deterioration of flavour and odour of fat or the fatty portions of foods. Such deterioration can be due to hydrolysis, oxidation, or to microbial action. The term rancidity is used in the dairy field to indicate hydrolytic deterioration; in other fields it denotes microbial deterioration, and to the fat chemist it means autoxidation. Only this latter type will be discussed here. For a discussion of other types of rancidity the reader is referred to a book by Lea.[1]

Autoxidative rancidity of fats is caused primarily by the attack of oxygen upon unsaturated constituents. The rate of uptake of oxygen by a fatty substance increases with the degree of unsaturation of the substance, and the mechanisms of oxidation for the various types of fatty acids are different. This chapter will concern itself with the mechanisms of autoxidative attack on fats and fatty acids, detection of oxidized products, and the effects of such oxidation upon the biological value of the fats. The mechanisms of oxidation in edible and drying oils are similar in the preliminary states, and will be treated together here, but the polymerization at advanced states of oxidation in drying oils will be treated in another chapter.

It is interesting to note that many of the facts concerning autoxidation were recognized as early as 1827 when Berzelius[2] described an experiment illustrating induction period, oxygen uptake, CO_2 formation, and polymerization:

> Among the changes which oils undergo, they take up oxygen from the air in amounts several times their volume. De Saussure reports that a layer of walnut oil which he left over mercury under oxygen took up three times its volume in 8 months, whereafter a more vigorous absorption began, so that within ten days it had taken up 60 times its volume, later diminishing and stopping after 3 months when the oil had absorbed 145 volumes of oxygen. The stronger absorption began at the beginning of August, in which there was a higher room temperature. No water was formed, but 21·9 times the oil's

volume of carbon dioxide was produced, the oil changed in an anomalous way, changed to a gelatinous mass and no longer gave an oil spot on paper. Walnut oil belongs to the drying oils. We have a corresponding example in the elevated temperature which develops when wool is lubricated with linseed oil, also belonging to the drying oils. If left in a heap, it often ignites itself, and in that manner it has destroyed many textile mills. Surely such a rapid absorption of oxygen is the cause of the elevated temperature.

Even now, in a field as large, as immature, and as controversial as this one, brief treatment as desired here can only include trends of thinking and some supporting evidences. Some trends of thought on autoxidation theory are supported by limited evidence, and are based largely upon the theory of organic chemistry. The theories discussed and mechanisms presented should be recognized as in a state of development, and should not be considered as fact. They are presented to stimulate discussion and investigation.

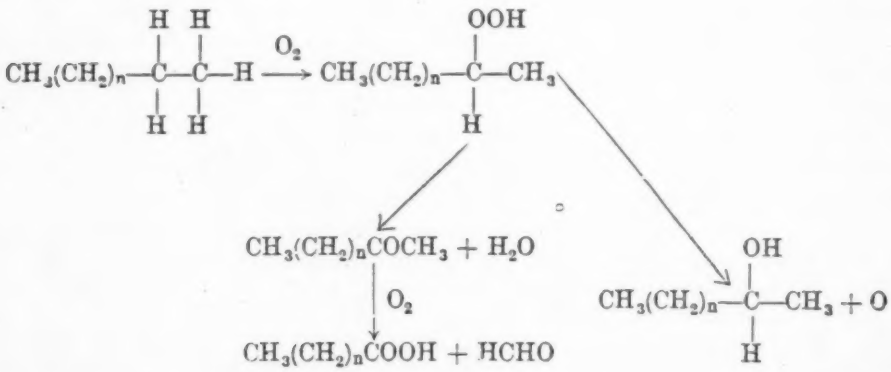
Mechanism of Autoxidation

Saturated compounds

Although it is generally recognized that the main problem of autoxidation of fats lies in the oxidation of their component unsaturated fatty acids, it has been demonstrated that saturated fatty acids do undergo a slow autoxidation, perceptible at elevated temperatures. The autoxidation of saturated hydrocarbons has been of considerable importance in lubrication problems, and information gained in numerous such studies is probably applicable to saturated fatty acids.[3]

At temperatures above 100°C, saturated normal hydrocarbons are subject to autoxidation. The attack is predominantly at a β-carbon and the primary oxidation product is postulated to be a hydroperoxide. Decomposition of the hydroperoxide can proceed by dehydration to yield a ketone, by reduction to yield an alcohol, or by further oxidation leading to rupture of the carbon chain.

These mechanisms are as follows:



Attack can also be made at other carbon atoms, giving rise to other species of compounds. The aldehydes, ketones, and acids among the reaction products are subject to further oxidation and polymerization. As an example giving some conception of the rate of these oxidations, cetane absorbs approximately

0·002 mol oxygen per hour at 110°.[4] There is a slight increase in rate of oxidation with increased chain length. Branched and normal paraffins show similar autoxidation curves, having typical induction periods. The reaction is autocatalytic and is subject to acceleration by metallic ions and to inhibition by either metal-binding compounds or by antioxidants. The reaction is believed to be a chain reaction. At 100° the oxidation of methyl stearate is one-eleventh as fast as that of methyl oleate.[5] With saturated fatty acids, increased chain length increases susceptibility to autoxidation.[6]

In a study of the oxidation of saturated acids in the presence of 0·1% $KMnO_4$ at 150°, it was found that lauric acid underwent no change, whereas stearic acid was decomposed to yield lower fatty acids, hydroxy compounds, and lactones.[7] Ethyl palmitate and ethyl caprate blown with air at 120° in the presence of 1% nickel phthalocyanine lost a small amount of weight and the exhaust air contained lower acids.[8] The residues contained lower acids and the refractive indices increased as the result of oxidation. Sodium or potassium soaps of fatty acids also act as catalysts for the oxidation of long chain acids.[9] Because the principal products of oxidation are oxalic acid and fatty acids of shorter chain length having even numbers of carbon atoms, the major attack appears to be by β-oxidation. However, the isolation of small amounts of latcones indicates some γ- and δ-oxidation. It is interesting to observe that β-oxidation, which is the predominant mechanism of metabolism of saturated acids, is also a chief mechanism of their autoxidation, whereas primary oxidation of poly-unsaturated acids proceeds by another mechanism, whether the oxidation is metabolic or autoxidative. For both types of acids, the similarity between metabolism and *in vitro* autoxidation is striking.

Monoethenoid compounds

The study of autoxidation of unsaturated compounds has a voluminous literature, and for a more detailed appraisal of the older literature the reader is referred to many reviews.[1], [10–16]

The autoxidation of monoethenoic fatty acids such as oleic acid, although autocatalytic, is relatively slow at ordinary temperatures, and catalysts, radiation, and elevated temperatures have been used to produce reasonable rates of oxidation for study. The rates of oxidation of oleic acid and ethyl oleate, for example, are respectively about 0·08 and 0·04 mol oxygen per mole substrate per 100 hr at 37° without catalyst.[17]

The older theories of fat oxidation involved the formation of a four-membered ring peroxide, or moloxide, as the primary reaction product:

$$-CH=CH- \xrightarrow{O_2} -\overset{O-O}{\underset{|}{CH}}-\overset{|}{CH}-$$

This reaction mechanism had its basis primarily in rather crude experiments in which the results indicated a decrease in total unsaturation concurrent with an increase in peroxide value during oxidation. In spite of considerable more

carefully gathered evidence to the contrary, and although a ring peroxide has never been isolated from the oxidation products of unsaturated compounds, this mechanism has persisted and appears in modern texts and reference works as the primary mechanism of autoxidation.

Recent reports, although not discarding this as a possible mechanism, have brought forth strong evidences for the existence of another type of peroxide, a hydroperoxide, in which the original double bonds remain. Current thought on the mechanism of autoxidation of monoethenoid compounds is a fusion of the older theory of attack at the double bond and the newer concept of attack at the α-methylenic carbons (those adjacent to carbons involved in the double bonds).

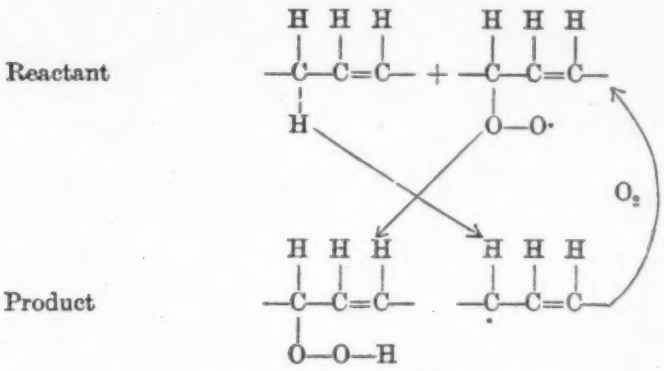
The early studies of reaction mechanism concentrated on isolation of reaction products. Oleic acid oxidized at 100–120° was found by SKELLON[18] to yield two 9,10-dihydroxy stearic acids, a monohydroxy stearic acid, and numerous compounds arising from chain scission. In studies by ELLIS,[19], [20] oxidation of oleic and elaidic acids was allowed to proceed until 1 to 3 mol of oxygen had been absorbed at 55–80° with cobaltous elaidate catalyst. Epoxide formed at least 20% of the product when catalyst was used. This was taken as evidence of oxygen attack at the double bond. Scission products included nonanoic, octanoic, suberic, azelaic, and oxalic acids in addition to carbon dioxide and water. Peroxide was a minor product. These results were confirmed in the main by DEATHERAGE and MATTILL[21] who found oxido derivatives to be the chief autoxidation products. The oxido-stearic acid formed by autoxidation of oleic acid appeared as half esters of dihydroxystearic acid and corresponded to the high melting dihydroxystearic acid isomer. In a study by SWERN *et al.*,[22] molecular distillation was used to fractionate products, and the product was found to contain polymers of approximate molecular weight 1700.

The oxidation of mono-unsaturated compounds is accompanied by changes in the ultraviolet spectrum.[23] As the oxidation proceeds, the light absorption increases in the regions near 2350 Å and 2700 Å, and the chromophore absorbing at the latter wavelength is alkali labile. Ketols, α-dicarbonyls, dihydroxy or epoxy compounds do not account for the spectral changes. It has been suggested that the light absorption is due to conjugated unsaturated ketones.

When oxidation was conducted at the relatively low temperature of 37° with a cobalt catalyst, FRANKE and JERCHEL[24] found less than half of the oxygen in the form of peroxide. At 35° under ultraviolet light and oxygen, the oxidation can be speeded up to 0·1 mol oxygen per mole oleate in 5 hr, at which time all oxygen is present as peroxide. FARMER and SUTTON,[25] using this technique, isolated nearly pure methyl hydroperoxido-octadecenoate by molecular distillation. Catalytic hydrogenation yielded a mixture of monohydroxy stearates, whereas aluminium amalgam reduction yielded a mixture of unsaturated hydroxystearates, thus demonstrating the presence of the double bond in the peroxide. SWIFT *et al.*[26] have confirmed this work by the isolation of 90% pure methyl hydroperoxido oleate by low temperature crystallization of oxidized methyl oleate. Oxidation of methyl elaidate under similar conditions also yields hydroperoxide products.[27]

Mechanism of Autoxidation

The older theory in which a four-member ring peroxide is the primary oxidation product has been largely supplanted in recent years by a theory of autoxidation developed in England.[28], [29] Autoxidation of all unsaturated substances proceeds by a free radical mechanism in which the primary point of attack is the α-methylene group. The oxidation proceeds by a chain reaction initiated by rer oval of a hydrogen atom from an alpha-methylene group

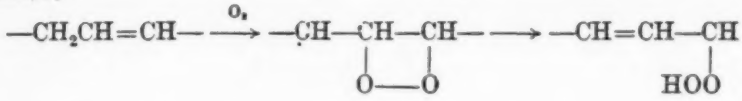


The postulated free radicals should exist as resonance hybrids, and the oxygen could thus attack at the 8, 9, 10, or 11 positions giving rise to four isomeric hydroperoxides.

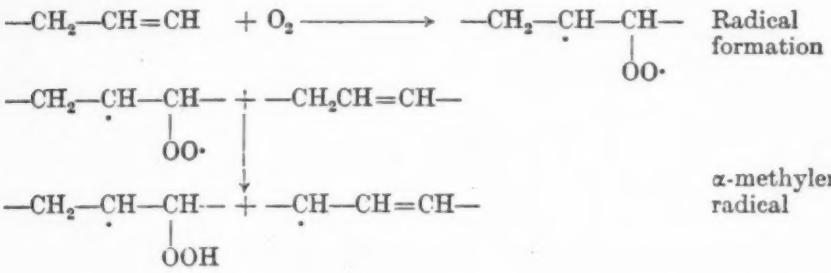
Ross *et al.*[30] examined by chemical means the hydroperoxides resulting from oleate oxidation under ultraviolet irradiation and concluded that substitution had occurred at all four positions. In a study of autoxidation of methyl stearolate, oleate, and 9,10-dideutero-oleate KHAN *et al.*[31] found fundamental differences between autoxidation of acetylenic and olefinic linkages. They demonstrated that some of the hydrogen arising during the oxidation came from the olefinic hydrogen, and came to the conclusion that oxidative attack is initiated at the double bond and then subsequently at alpha-methylenic positions. MAX and DEATHERAGE[32] found that oxidation of 8, 8, 11, 11-tetradeutero-*cis*-9-octadecene proceeds one-fifth as rapidly as that of *cis*-9-octadecene, indicating the importance of oxidative attachment at the α-methylenic positions in sustaining the reaction. The low deuterium content of the water indicated that it is not formed by decomposition of the α-hydroperoxides.

From examination of the isolated hydroperoxide, SWIFT *et al.*[26] concluded that it was chiefly 8 and 11 hydroperoxido-octadecenoate. Hydroperoxide was found to oxidize oleic acid at 90° to yield epoxides and the low melting form of 9,10-dihydroxy stearic acid.[33] Such a reaction between oleate and its primary oxidation product could be a factor in the reduction in total iodine value observed in autoxidizing fats and the formation of epoxides and dihydroxy stearates, formation of which is often taken as evidence of double bond attack. Decomposition of methyl hydroperoxido-oleate proceeds by scission to yield α,β-unsaturated carbonyl compounds, among which 2-undecenal has been shown to occur.[34]

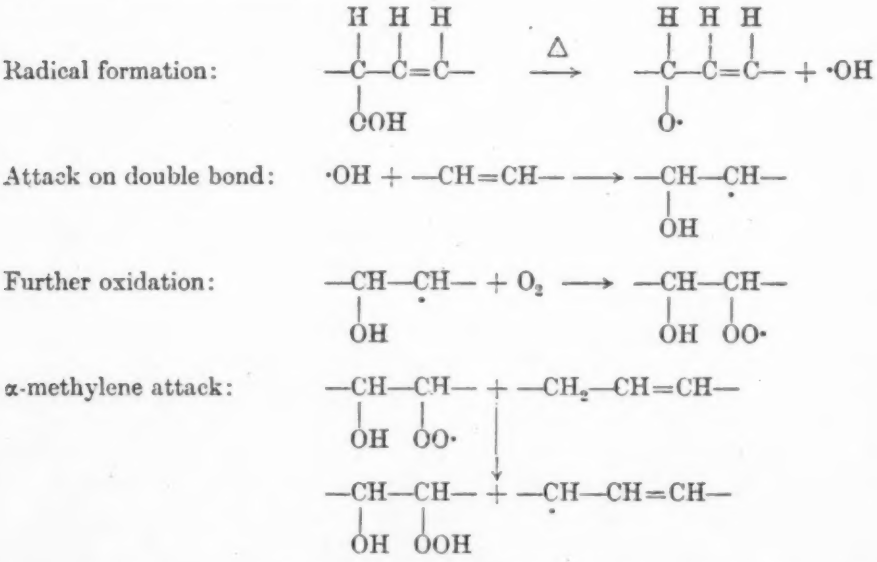
HILDITCH[35] has proposed the addition of oxygen directly to the double bond as the primary step followed by a rearrangement to give a hydroperoxide. This mechanism,



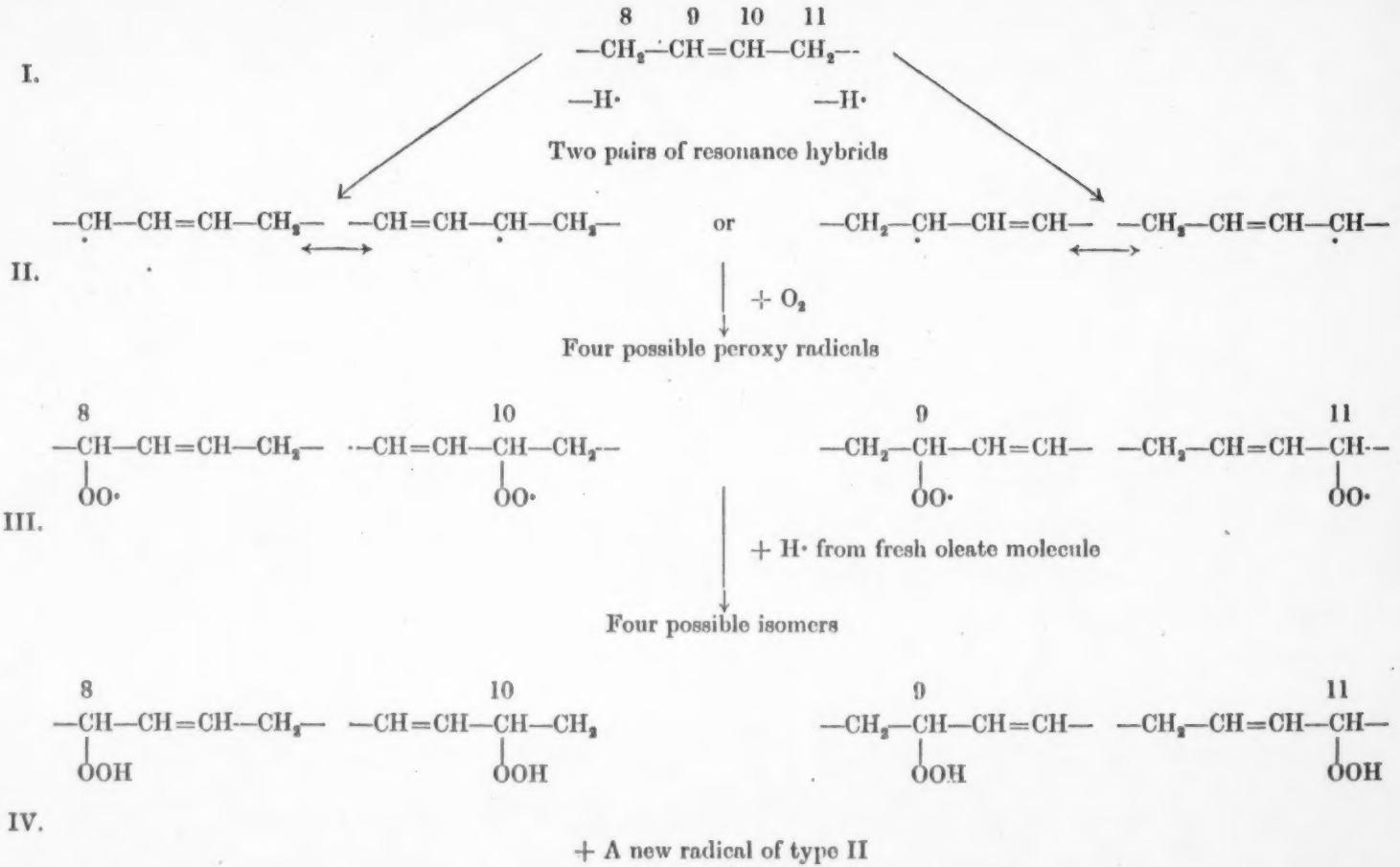
would lead to 9 and 10 hydroperoxides but no 8- or 11-isomers, such as reported by other workers. However, possibility exists that the mechanism of addition to the double bond yields radicals which then propagate the reaction through α-methylene attack.



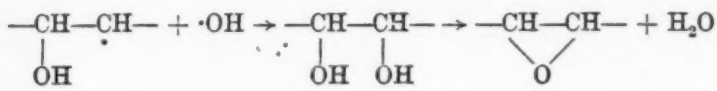
Another mechanism has been proposed to account for the loss of double bonds in autoxidation at high temperatures[12] based upon observations that hydroperoxides undergo thermal decomposition yielding hydroxyl radicals which apparently add to double bonds.



The peroxide formed by the latter reaction and that formed by subsequent reaction of the last free radical with oxygen could then presumably both yield


I.
8 9 10 11
—CH₂—CH=CH—CH₂—
—H·
—H·
Two pairs of resonance hybrids
II.
—CH—CH=CH—CH₂— ⟷ —CH=CH—CH—CH₂—
or
—CH₂—CH—CH=CH— ⟷ —CH₂—CH=CH—CH—
+ O₂
Four possible peroxy radicals
III.
8
—CH—CH=CH—CH₂—
OO·
10
—CH=CH—CH—CH₂—
OO·
9
—CH₂—CH—CH=CH—
OO·
11
—CH₂—CH=CH—CH—
OO·
+ H· from fresh oleate molecule
Four possible isomers
IV.
8
—CH—CH=CH—CH₂—
OOH
10
—CH=CH—CH—CH₂
OOH
9
—CH₂—CH—CH=CH—
OOH
11
—CH₂—CH=CH—CH—
OOH
+ A new radical of type II

hydroxyl radicals to propagate the chain. Epoxides and glycols could be explained by chain stopping addition of the two radicals:

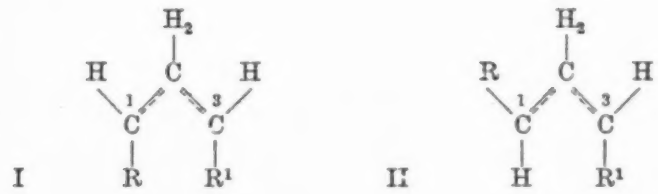


Subsequent oxidation of these compounds could lead to rupture of the carbon chain.

Autoxidation of a terminal double bond may involve a different mechanism. 10,11-undecylenic acid oxidized at 80° by air yields sebacic acid as chief product, but some 10,11-dihydroxy hendecanoic acid and polymers are formed.[36]

The favoured mechanism of autoxidation of oleate dominant at ordinary temperatures in the initial stages of oxidation can be represented as on p. 57. This mechanism is probably supplanted by other mechanisms involving attack at the double bond at later stages of the oxidation, at higher temperatures and in the presence of catalyst. These conditions are conducive to peroxide decomposition, and the hydroxyl radicals formed alter the course of the oxidation to one in which the dominant attack is at the double bond.

Recently it has been demonstrated by infrared observations that in the early stages of autoxidation of methyl oleate under ultraviolet light, peroxide formation is accompanied by the appearance of *trans* double bonds in an amount approximately 90% of the peroxide formed.[37] Among the possible mechanisms for this isomerization, the following mechanism was given. In the free radical formed, the atoms probably lie in a plane providing maximum resonance energy. The radical could then have two isomeric forms:



Addition of oxygen to carbon 1 of either I or II or carbon 3 of I would yield a *cis* hydroperoxide. Infrared observations suggest that most of the radicals assume the configuration II and add oxygen to carbon 3 yielding a mixture of *trans*-octadecenoates.

Much of the confusion concerning which is the primary oxidation product can be explained by the results of an analytical study of oleate oxidation by KNIGHT *et al.*[38] Their results (Fig. 1) show striking differences in the course of the reaction at different temperatures. Samples taken for analysis at different stages of oxidation would likewise lead to differing conclusions concerning the main products.

The results of this latter investigation and the previously-mentioned studies serve to demonstrate the complexity of the autoxidation reactions. It appears certain that numerous reactions take place in the process of autoxidation, and that the individual reactions are affected differently by temperature, catalyst, and other factors.

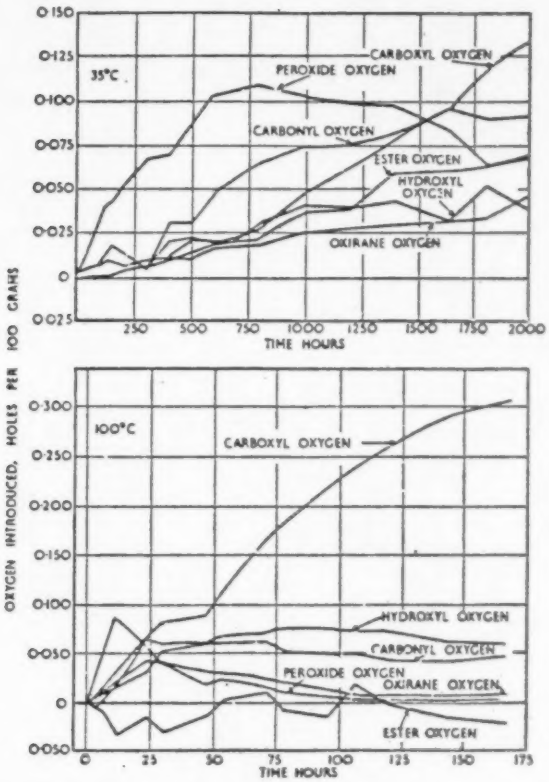


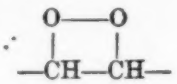
Fig. 1. Relationship of oxygen-containing functional groups in methyl oleate autoxidized at 35° and 100° (from KNIGHT *et al.*).[88]

Nonconjugated polyethenoid compounds

The rate of oxidation of methylene-interrupted poly-unsaturated systems is much higher than that of monoethenoic systems because of the activation of a methylene group by two adjacent double bands. This double activation results in oxidation rates twenty to forty times as great as in singly unsaturated compounds, making the polyethenoic acids the main source of oxidative rancidity problems.

Early work on autoxidation involved observations on changes in iodine value, peroxide number, refractive index, acid value, etc., which did provide sufficient information to develop a theory of autoxidation that has been held almost to the present. The observation that as oils are oxidized, their iodine values decrease,

was strong evidence for a mechanism in which double bond attack was the main feature. The conception of the ring peroxides or moloxides,



has persisted in spite of much evidence for the methylene attack and hydroperoxide formation. The reduction in iodine value in the early observations was due to several factors: (1) Drastic oxidation does reduce iodine value by com-

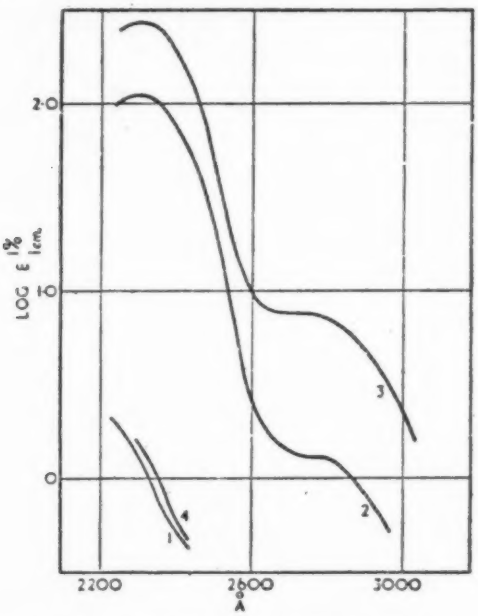


Fig. 2. Effect of oxidation upon ultraviolet spectrum of ethyl linoleate. (1) Pure ethyl linoleate. (2) After 1·6% oxygen uptake. (3) After 4·5% oxygen absorption. (4) After chromatographic removal of oxygenated fraction (from Bolland and Koch).[24]

plex side reactions and chain scission; (2) The conjugation produced during autoxidation of poly-unsaturated systems reduces apparent iodine value measured by conventional procedures because halogen does not add to conjugated systems as readily as to non-conjugated systems; (3) Iodine value measurements are low in the presence of oxidized fats, possibly because of steric hindrance to halogen addition.

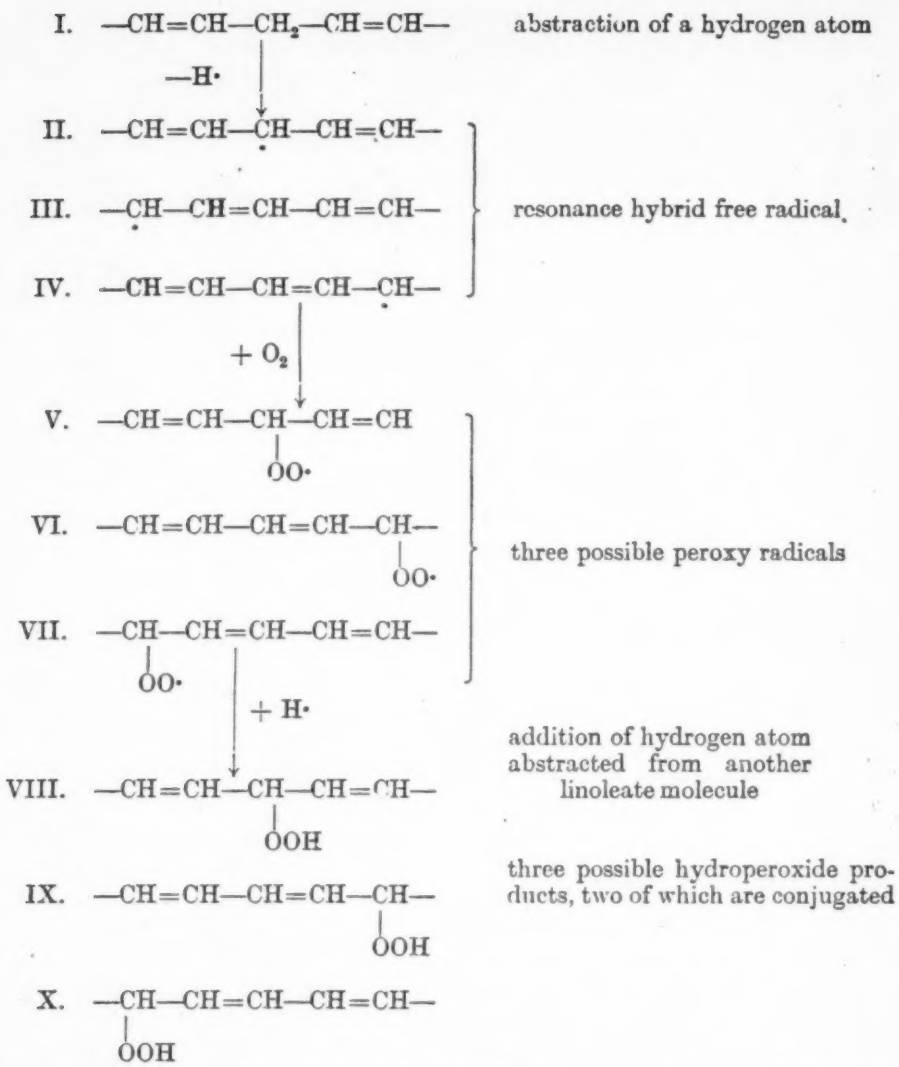
It was not until 1943 when workers in the British Rubber Producers Research Association observed the conjugation of double bonds in autoxidizing fish-oil acids that the current theory of autoxidation began to develop.[39] When oils containing linoleate or more highly unsaturated systems are autoxidized, the diene conjugation as measured by ultraviolet light absorption at 2340 Å increases parallel with oxygen uptake and peroxide formation in the early stages

of oxidation. The spectra of fresh and oxidized linoleate are shown in Fig. 2.[36] That this light absorption is not due to the peroxide structure has been shown in an experiment in which thermal decomposition of peroxide did not diminish the light absorption.[23]

The spectral changes occurring in autoxidizing fatty materials have been independently observed by several investigators[23], [41–49] and have been studied in considerable detail. The spectral changes accompanying oxidation are qualitatively similar for fatty acids containing two or more double bonds interrupted by methylene groups. Oxidized linoleate has a principal absorption at 2300–2360 Å, due to diene conjugation, and a secondary smooth absorption maximum at 2600–2800 Å, probably due to small amounts of unsaturated ketones. The principal band is the same for linoleate, linolenate, and arachidonate, but the greater the degree of unsaturation, the lower the diene conjugation absorption per mole of absorbed oxygen. Conversely, the more unsaturated the fatty ester, the greater the light absorption caused by secondary reaction products. The light absorption in the longer wavelength range is considerably increased in alkaline solution.[23] This spectral shift is diphasic, consisting of an immediate reversible increase followed by a slow irreversible shift, suggesting enolization and condensation.[47] This intensification of colour (end absorption of chromophores absorbing in the ultraviolet region) is often encountered when rancid oils are saponified. Fresh oils and pure fatty acid preparations show no such spectral shift in alkali, nor do pure conjugated polyenes, indicating the dependence of this phenomenon upon oxygen-containing products. The spectral shift in alkali has been used with some success by HENDRICKSON *et al.* in discovering past oxidative treatment of drying oils.[50]

The group of investigators at the British Rubber Producers Association is largely responsible for developing the current theory of the mechanism of oxidation of poly-unsaturated substances. The numerous papers by FARMER, BOLLAND, KOCH, GEE, BATEMAN, SUTTON, and ORR on the kinetic theory and supporting chemical and physical evidences have been the chief contributions in evolving the modern concept of fat oxidation.[39], [40], [51–60]

The mechanism of reaction originally proposed by BOLLAND and KOCH[40] is on p. 62. The molecular extinction coefficient for linoleate hydroperoxide has been calculated from the light absorption and oxygen content of oxidizing linoleates during the initial stages of the oxidation. The highest value is 22,700 reported by BOLLAND and KOCH. This value is about 70% of the molar extinction coefficient of conjugated linoleic acid, and this was taken as supporting evidence for the concept of the random attack of oxygen on the free radical formed from linoleate, giving rise to products, two-thirds of which were conjugated diene hydroperoxide. However, BERGSTRÖM has chromatographed the hydrogenated products of linoleate oxidation and has isolated and identified 9- and 13-hydroxystearates.[46] He was unable to detect any 11-isomer such as would be produced if oxygen had attacked the resonance hybrid randomly. These results do not conclusively exclude the 11-hydroperoxide as a product of oxidation of linoleate,



because under the conditions of hydrogenation, rearrangement of the non-conjugated isomer to conjugated isomers could take place.

Aside from BERGSTRÖM's chemical evidence, only thermodynamic evidence has argued for the formation of conjugated hydroperoxide in more than random amounts.[51]* The resonance energies associated with the radical systems

* Recently KHAN, LUNDBERG and HOLMAN have obtained chromatographic evidence to support BERGSTRÖM[803]. Methyl linoleate was oxidized either by autoxidation in the dark at − 10°, under visible or ultraviolet light, or in the presence of copper catalyst. The peroxides developed were segregated by countercurrent extraction and then reduced by stannous chloride. These hydroxy-linoleates were then separated by displacement chromatography. In the above-mentioned examples the products consisted almost entirely of conjugated compounds, but in the case where oxidation was stimulated by chlorophyll and irradiation a non-conjugated product was isolated. These observations suggest strongly that the conjugation observed was not induced by the reduction, and that autoxidation results largely in conjugated products.

R—CH=CH—ĊH—CH=CH—R and R—CH=CH—ĊH—R have been calculated by BOLLAND and ORR[52] and are estimated to be 30·5 and 18·7 kcal per g mol respectively. Thus the formation of the unconjugated type radical is much less probable than the conjugated type. BOLLAND[51] states that "although the position into which substituents (e.g. —OOH) are introduced into the radical by succeeding reaction processes may well be affected by other factors, it is probably of significance that the formation of products of the type (IX) and (X) will be favoured at the expense of (VIII) by resonance energy (*ca.* 7 kcal per g mol) associated with their conjugated structure." Moreover, BOLLAND points out that from thermodynamical considerations the probability of oxidative attack on linoleate hydroperoxide leading to diperoxides is small in the early stages of oxidation.

A resonating radical cannot be described adequately by graphic means. The three structures, II, III, and IV, represent the two extremes and a median structure which exist only for infinitesimal periods of time. The true structure of the radical would be represented by the statistical distribution of electron densities about the five carbon atoms involved. In this system the resonance is such that higher electron densities occur at the ends of the system a large proportion of the time. Thus when this radical is attacked by another radical or oxygen the probability that the addition occurs at the end of the system is higher than would be expected by random attack. Thus the hydroperoxide formed in linoleate oxidation would be greater than two-thirds and less than completely conjugated. The extent of conjugation occurring during linoleate oxidation is now the subject of active research.

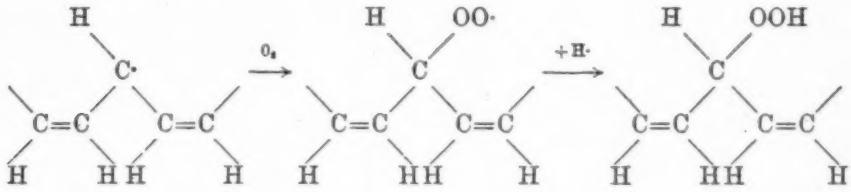
Much of the uncertainty regarding the true extent of conjugation produced arises from lack of proper standards of comparison. The estimates in the literature are based upon comparison with *trans, trans* 10,12-octadecadienoic acid which has a molar extinction coefficient of about 32,000. However, the effect of the peroxide group in the molecule, and the effect of *cis trans* isomerism were not taken into consideration in making these comparisons.

Recent infrared spectral studies on the *cis trans* isomers of linoleic acid, reviewed by WHEELER in another chapter in this volume, indicate that the conjugated linoleate hydroperoxide is not *trans, trans* and that estimates of the degree of conjugation present in the product must be revised upward. Recent studies of conjugated linoleate isomers obtained by alkali isomerization of linoleate indicate that these isomers have lower extinction coefficients at 2320 Å than the better known *trans, trans* isomers.[61], [62] PRIVETT *et al.*[63] have recently studied the infrared spectra of linoleate hydroperoxide preparations of very high purity and have found that the product is at least 90% conjugated and that it consists largely of *cis, trans* isomers. The degree of conjugation calculated from ultraviolet absorption of the peroxide and known conjugated *cis, trans* linoleate also indicated at least 90% conjugation. These high degrees of conjugated *cis, trans* hydroperoxide were found only in preparations oxidized near 0°C. Oxidation at 24°C yielded peroxide concentrates in which appreciable amounts of conjugated *trans, trans* forms existed. The authors suggested that

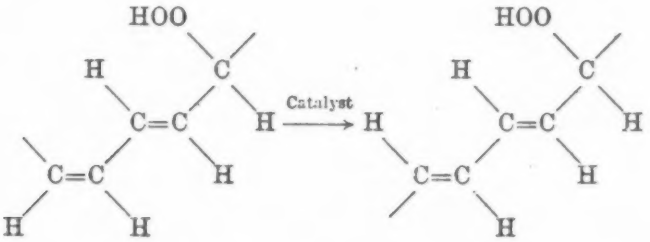
conjugated *cis, trans* isomers were initially formed and that the thermodynamically more stable conjugated *trans, trans* isomers arose from them through some catalysis, possible by peroxides.

In the light of the foregoing observations, the simplified mechanism on p. 65 for the main course of linoleate autoxidation is offered.

Linoleate, I, loses a hydrogen atom to some radical and becomes a free radical II. This free radical is a resonance hybrid, the two extreme forms of which are shown as III. Oxygen adds to the resonating radical, predominantly at the ends of the resonating system to yield two types of hydroperoxy radicals, IV. These radicals accept hydrogen atoms from other linoleate molecules to become isomeric conjugated *cis, trans* hydroperoxides, and in so doing perpetuate the cycle. Other reactions which occur to a limited extent under ideal conditions should be mentioned: (1) Oxygen may add to the intermediate form of the free radical II to yield non-conjugated peroxides.



(2) The conjugated *cis, trans* linoleate peroxide may be isomerized to a *trans, trans* form:



(3) Polymers may be formed by addition of radicals II, III, IV with each other:

$$ROO\cdot + R\cdot \rightarrow ROOR$$
$$R\cdot + R\cdot \rightarrow R{-}R$$

The cyclic or chain nature of the reaction is well established. The entire mechanism, however, involves three types of reactions: chain initiation, chain propagation, and chain stopping reactions. The reaction chain can be initiated by the attack of any free radical upon linoleate. The most probable radicals to initiate chains are those formed by decomposition of a peroxide. It was formerly believed that peroxide was not required for chain starting, for BOLLAND had shown that ethyl linoleate had a low but measurable rate of oxidation at 0% oxidation.[51] However, LUNDBERG *et al.*[47] demonstrated that highly purified linoleate had a long induction period, that is, it had no measurable oxidation

(I.)
(II.)
(III.)
(IV.)
(V.)
OR
O_2
+H·
−H·

rate for several hours after exposure to oxygen. BATEMAN has recently communicated privately that the rate of oxidation at zero degree of oxidation is apparently zero. The first chain must thus be initiated by some nonperoxidic free radical or by stray radiation. Parallel oxidation chains are initiated by radicals formed by decomposition of hydroperoxide. The higher the concentration of peroxide the more rapid is its rate of decomposition.[64] Thus as the oxidation proceeds it generates its own catalyst. Hence the autocatalytic nature of the reaction. The reaction chains can be stopped by collision of two radicals, for example:

$$R\cdot + R\cdot \rightarrow RR$$

$$R\cdot + ROO\cdot \rightarrow ROOR$$

$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$$

If the radicals attack molecules of other substance present, products may be formed which do not decompose to form radicals and are thus incapable of propagating the reaction chain. Such other substances are thus antioxidants (*vide infra*). For more detailed discussions of the kinetics of oxidation, see the papers of BOLLAND *et al.*[40], [51], [52], [55], [57], [60] and WATERS.[65] The reader is also referred to the work of HILDITCH[66] whose conception of the reaction mechanism is not in agreement with that presented here, and to GIBSON who presents the mechanism of oxidation in a rather unique manner.[67]

Conjugated polyethenoid compounds

Although the conjugated fatty acids present in tung oil, oiticica oil, and dehydrated castor oil are of great importance in compounding protective coatings, the oxidation of these substances has received less study than that of the non-conjugated fatty acids. The mechanism of oxidation of these substances is considerably different from that of the non-conjugated isomers, and the products of the reaction are also different.

Much of the pioneering in this area was done by MORRELL and his co-workers who studied the oxidation of eleostearate and its maleic anhydride adducts.[68], [69] MILLER and CLAXTON[70] made a thorough study of the physical and chemical changes occurring during the course of the oxidation of methyl and glycol esters of β-eleostearic acid at elevated temperatures. Their results indicated increased molecular weight, saponification number, acid number, and specific gravity during the course of the reaction, accompanied by decreased refractive index, iodine number and *p*H. They concluded that oxygen-containing polymers were formed and that scission products contributed to the polymer. They also found evidence for ketol and enol groups in the oxidized product. BRAUER and STEADMAN[71] studied the course of oxidation of β-eleostearic acid by means of spectrophotometric measurements. They observed that the light absorption in the 2600–2800 Å region due to conjugated triene, decreased as the oxidation proceeded and that absorption due to conjugated diene increased. This has been found to be true also for pseudoeleostearic acid, α-eleostearic, and

β-licanic acid.[42] Fig. 3 shows the changes that occur during oxidation of pseudoeleostearic acid (10,12,14-octadecatrienoic acid).

The action of alkali upon oxidized conjugated trienes causes strong increases in absorption in the longer wavelengths suggesting the presence of enolizable substances in the oxidized mixture.

ALLEN, JACKSON, and KUMMEROW[72] compared the oxidation of 9,12 and 10,12 methyl linoleate and found that in the early stages of oxidation of 9,12 linoleate, all the oxygen absorbed was found as peroxide, whereas in the oxidation of the conjugated isomer, no peroxide accumulated in the first stages of the

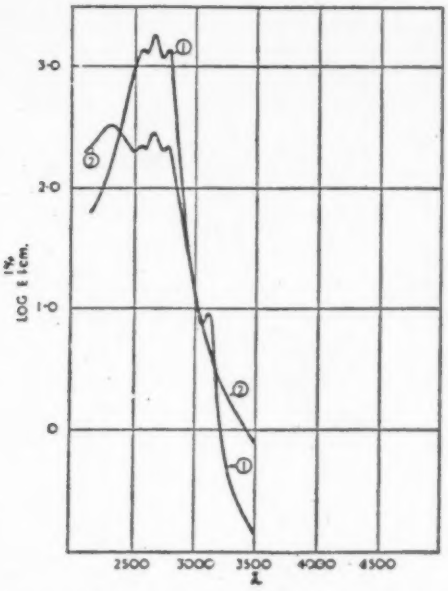


Fig. 3. Effect of oxidation on ultraviolet absorption spectrum of pseudoeleostearic acid. (1) Fresh pseudoeleostearic acid. (2) In air at 77° for 6 hr.

process. The disappearance of conjugated double bonds was equivalent to oxygen absorbed, suggesting that carbon to oxygen polymerization occurs rather than carbon to carbon polymerization. The work of JACKSON and KUMMEROW[73] on oxidation of the two linoleate isomers in the presence of metallic naphthenate driers indicates that the driers had less effect upon the oxidation of conjugated linoleate than upon non-conjugated linoleate. This would also suggest that peroxide decomposition is not a major factor in the mechanism of oxidation of conjugated substances.

The oxidation of conjugated unsaturated substances is accompanied by less breakdown than is the oxidation of non-conjugated substances. The conjugated triene fatty acids and esters oxidize at a faster rate than do their non-conjugated isomers. This was shown by the work of MYERS, KASS, and BURR (Fig. 4) who compared the oxidation of small amounts of trienoic acids and esters on filter

paper.[74] However, comparison of the rates of oxidation of linoleic acid and 10,12 linoleic acid showed no essential difference.[17] On the other hand, oxidation of the conjugated methyl linoleate proceeded slower than oxidation of methyl linoleate in the experiments reported by ALLEN *et al.*[72]

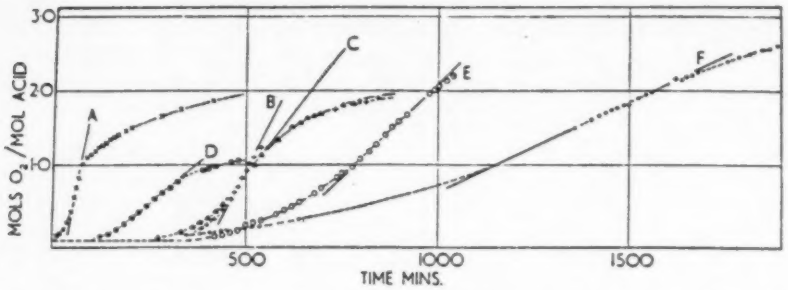


Fig. 4. The course of oxygen absorption by trienoic fatty acids and esters at 40°. (*A*) α-eleostearic acid. (*B*) β-eleostearic acid. (*C*) Pseudo-eleostearic acid. (*D*) Methyl pseudo-eleostearate. (*E*) Linolenic acid. (*F*) Ethyl linolenate (from MYERS *et al.*).[74]

The most thorough report on eleostearate oxidation is that of ALLEN and KUMMEROW.[75] They found that the amount of triene conjugation lost and the amount of diene conjugation formed were both proportional to oxygen absorbed (Fig. 5). The primary product of oxidation was isolated by low temperature

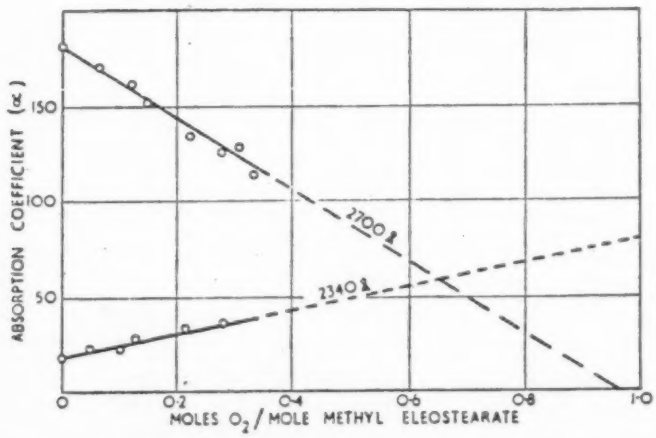


Fig. 5. Decreased triene conjugation and increased diene conjugation during the autoxidation of methyl eleostearate (from ALLEN and KUMMEROW).[75]

crystallization and found to possess strong conjugated diene absorption in the ultraviolet. The effect of alkali upon this absorption was minimal. Hydrogenation of this oxidation product yielded mostly methyl dihydroxystearates. Oxidation of these with alkaline permanganate yielded only valeric and azelaic acids, indicating that the original oxidative attack had been confined within

the triene system. The attack of oxygen upon the conjugated system was postulated to be 1,2, 1,4, or 1,6, yielding the following possible partial structures:

I. $R—CH=CH—CH=CH—\dot{C}H—\dot{C}H—R$

II. $R—\dot{C}H—CH=CH—\dot{C}H—CH=CH—R$

III. $R—\dot{C}H—CH=CH—CH=CH—\dot{C}H—R$

Two of these three possess residual diene conjugation. The isolated primary product of oxidation had a specific extinction coefficient of 62, compared with a value of 71 obtained by calculation. Upon hydrogenation at least three isomeric dihydroxy-stearates were obtained, only one of which contained an α,β-dihydroxy group. In accord with these chemical data and a kinetic study of the reaction, the following mechanism was proposed:

$$CH_3(CH_2)_4CH=CH—CH=CH—CH=CH—(CH_2)_7COOH + O_2 \rightarrow$$

$$CH_3(CH_2)_4CH=CH—CH=CH—\dot{C}H—\underset{\displaystyle OO\cdot}{\underset{|}{CH}}—(CH_2)_7COOH$$

This diradical is stabilized by resonance along the unsaturated system, allowing addition at any of the carbons of the triene system. Reaction with another unsaturated molecule would yield a dimer still possessing free radical centres:

$$—CH=CH—CH=CH—\dot{C}H—\underset{\displaystyle OO\cdot}{\underset{|}{CH}}— + R—CH=CH—R \rightarrow$$

$$—CH=CH—CH=CH—\dot{C}H—\underset{\displaystyle R—\dot{C}H—\underset{\displaystyle O}{\underset{|}{CH}}—O}{\underset{|}{CH}}—$$

This dimeric diradical could stabilize itself internally to give a cyclic peroxide:

$$—CH=CH—CH=CH—\underset{\displaystyle R—CH\quad\quad O\quad\quad CH—O}{\underset{|}{CH}}—\underset{\displaystyle R}{\underset{|}{CH}}$$

However, the dimeric radical could again add oxygen and olefin thus building a polymeric chain in which the repeating unit is —CHR—CHR—OO—. Oxidation of pure material would favour the latter course whereas dimer formation

would be favoured in diluted media. The kinetic studies allow the rate of reaction of eleostearate oxidation to be summarized by the equation:

$$dO_2/dt = K(\text{product})^{\frac{1}{2}}(\text{ester})$$

The rates of oxygen uptake for various conjugated unsaturated systems diminish markedly when the total oxygen uptake approaches 2 mol oxygen per mole ester or acid. This is true even for α- and β-parinaric acids which possess a conjugated tetraene system.[76] Apparently the polymer formation increases the steric hindrance making further oxidative attack difficult. Increasing viscosity and diminished diffusion of oxygen is apparently a lesser factor, for CHIPAULT *et al.*[77], [78] found that trieleostearin, pentaerythritol eleostearate and an eleostearic alkyd all became hard at a very early stage of oxidation, long before the maximum oxygen uptake had been achieved. On the other hand, the films set and hardened at later stages in the oxidation of similar linoleate and linolenate compounds.

The reaction mechanism quoted above does not account for all the observations concerning conjugated polyene oxidation. The oxidation of the eleostearates has been shown to be autocatalytic both by BRAUER and STEADMAN[71] in solutions, and by MYERS *et al.*[73] in thin films. According to the diradical reaction mechanism of KUMMEROW and his associates, propagation of the chain reaction is by polymerization. The formation of small molecule products such as the dimers found by BRAUER and STEADMAN is by early termination of the reaction chains. Chain reaction leading to monomeric or dimeric products could be only via a monoradical mechanism. Such a mechanism would involve the abstraction of a hydrogen from an eleostearate molecule.

The mechanism as postulated does not provide for auto-catalysis. The chain polymerization reaction is not autocatalytic. Moreover, it is implied that polymerization is primarily through carbon to oxygen bonds, whereas it is known that eleostearate sets a hard film very early in the process of oxidation—before sufficient oxygen has been absorbed to account for the cross linking. Thus the polymerization is probably mostly through carbon to carbon linkages. It does not appear that the free radical reaction mechanism for conjugated polyene oxidation is obligatory. The simple 1,2 or 1,4 addition of oxygen may account for many of the facts, except that it does not account for autocatalysis. It would explain the partly conjugated primary product of oxidation isolated by ALLEN and KUMMEROW, and early polymerization could be stimulated by peroxide catalysis. The reaction mechanism is by no means settled, and it is hoped that this discussion will stimulate investigations to learn its true mechanism.

Acetylenic compounds

Although acetylenic fatty acids do occur in natural oils and these substances are subject to autoxidation, very little information has been gained regarding the mechanism of this oxidation. KHAN, DEATHERAGE, and BROWN[79] have made a study of the autoxidation of stearolic acid and its methyl ester in comparison with oxidation of oleates. In contrast to oleates, stearolic acid and methyl

stearolate have no induction period. The oxidation begins at its maximum rate. The acetylenic compounds absorb oxygen at a faster rate than the oleates. This is illustrated in Fig. 6. Oxidation of stearolate was accompanied by considerable polymerization and the residues contained a considerable amount of carbonyl oxygen. No diketostearic acid was found, however. Acid and ester groups were present but only small amounts of peroxide and hydroxyl groups were present. The volatile products of oxidation of stearolate consist of water, carbon dioxide, and other organic products. In the case of methyl stearolate, water evolved equalled more than 0·8 mol per mole substrate and carbon dioxide more than

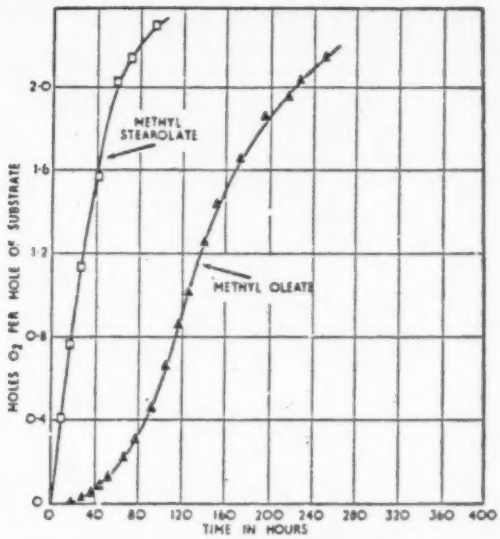


Fig. 6. Oxygen absorption of methyl oleate and methyl stearolate at 75° (from KHAN *et al.*).[79]

0·1 mol per mole substrate. The nonaqueous volatile products of stearolate oxidation had a rancid odour but gave a negative Kreis test, whereas the corresponding product from oleate gave a positive test. These striking differences between the kinetics and products of oxidation do strongly indicate a difference between the mechanism of oxidation of oleate and stearolate. KHAN *et al.* suggest that α-methylene attack of acetylenic compounds is the predominant type of oxidation rather than addition to the double bond.

A study of the oxidation of matricaria ester (n-decadiene-2,8-diyne-4,6-oic acid methyl ester) by HOLMAN and SØRENSON[80] indicates that in this conjugated system the oxygen addition follows a biphasic curve. Likewise, the increase in light absorption at 3550 Å in alkaline solution increases rapidly initially, whereas the increase in absorption at 6000 Å corresponds to the second phase of oxygen addition. The oxidation was accompanied by rapid polymerization and intense deepening of colour. The mechanism of oxidation of conjugated unsaturated systems involving triple bonds, such as occur in matricaria ester, and isano oil, is probably much different from that of isolated triple bonds. It is likely that

the mechanism of oxidation of these mixed double and triple bond conjugated systems is similar to that of conjugated polyenes, for rapid polymerization occurs during their oxidation. One would expect oxygen to add to the two types of resonating systems in similar manners.

Factors Affecting Rate of Oxidation

Degree of unsaturation

Although it is well known that the rate of oxidation of oils varies with the iodine value, the effect of increasing unsaturation is best shown in a series of purified

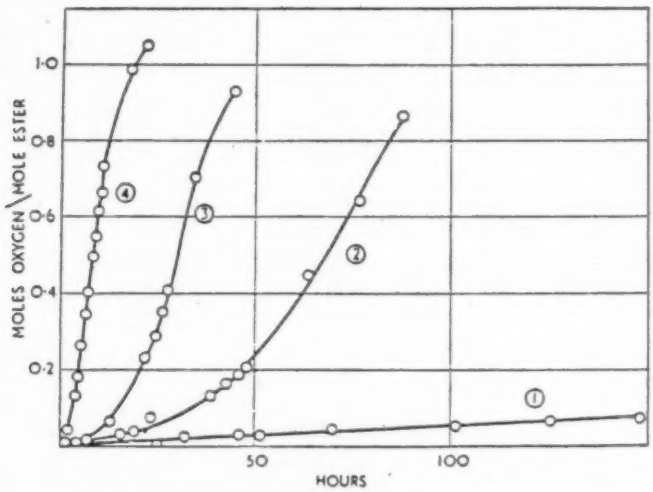


Fig. 7. Rates of oxidation of unsaturated fatty acid esters at 37°. (1) Ethyl oleate. (2) Ethyl linoleate. (3) Ethyl linolenate. (4) Methyl arachidonate (from Holman and Elmer).[17]

fatty esters, because with these, the complications of pro- and antioxidants are eliminated. With increasing number of isolated double bonds the maximum rate of oxidation increases.[17] The course of the oxidation of esters containing one through four isolated double bonds is shown in Fig. 7. The rates of oxidation at 25° and 37° for a series of esters is shown in the accompanying table. The large difference in rate of oxidation of oleate and linoleate is due to the great lowering of activation energy required for oxidation in the case where the methylene group is flanked on both sides by double bonds. The additional increases in rate of oxidation due to added numbers of double bonds is in the order of a factor of 2 per additional double bond. This generality was likewise true in a study by Chipault[81] of the oxidation of trilinolein and trilinolenin in the presence of cobalt-lead drier.

The effect of the presence of small portions of linoleate upon the rate of oleate oxidation has been studied by Gunstone and Hilditch.[82] The development of peroxide in oxidizing mixtures of oleate and linoleate is shown in Fig. 8. From these studies it is apparent that high purity of substrate is important in oxida-

tion studies. In his kinetic studies on linoleate oxidation, BOLLAND[57] likewise studied the effect of ethyl linoleate concentration in ethyl oleate. He observed

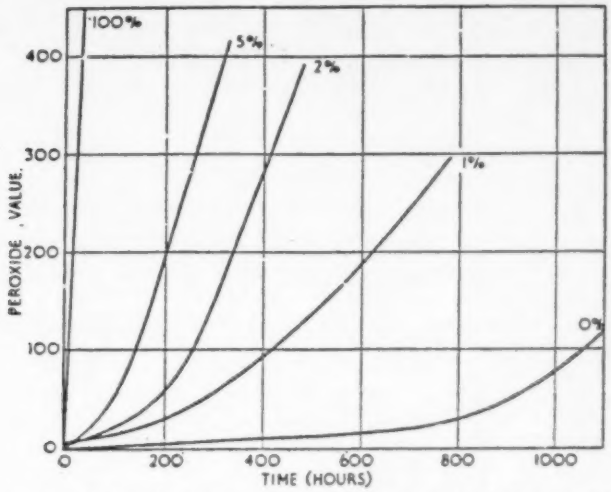


Fig. 8. Rates of oxidation of mixtures of methyl linoleate in ethyl oleate at 20°C. Figures refer to linoleate concentration (from GUNSTONE and HILDITCH).[83]

that the relative rate of oxidation bore a linear relationship to the molar concentration of linoleate. Thus the oxidation of the diluent, oleate, is not "catalyzed" by the presence of linoleate; the effect is one of dilution (*vide infra*).

Table 1. Maximum rates of oxidation of acids and esters

Substance	*Moles O_2/Mole acid/hour*	*Temperature*
Ethyl oleate[17]	0·0004	37°C
Ethyl linoleate[17]	0·0163	37°C
Ethyl linolenate[17]	0·0390	37°C
Methyl arachidonate[17]	0·0778	37°C
Methyl docosahexaenoate[80]	0·12	37°C
Oleic acid[17]	0·0008	37°C
Linoleic acid[17]	0·0220	37°C
Linolenic acid[17]	0·0618	37°C
Glycerol trilinoleate[81]	0·020	25°C
Glycerol trilinoleate*[81]	0·198	25°C
Glycerol trilinolenate[81]	0·048	25°C
Glycerol trilinolenate*[81]	0·40	25°C
Glycerol trieleostearate*[81]	0·50	25°C

* Cobalt lead drier added.

Conjugation

In compounds with more than two conjugated double bonds, conjugation increases the rate of oxidation. MYERS, KASS, and BURR[74] found that conjugated triene esters oxidized more rapidly than non-conjugated triene esters

(Fig. 4). CHIPAULT found that trieleostearin oxidized more rapidly than trilinolenin in the presence of drier (Table 1). However, ALLAN, JACKSON, and KUMMEROW[72] found conjugated methyl linoleate oxidized slower than methyl linoleate, and HOLMAN and ELMER[17] found the rates for linoleic acid and conjugated linoleic acids to be essentially the same.

Triple bonds

Only one study known to the author permits an evaluation of the effect of a triple bond upon the autoxidation of unsaturated fatty acids. KHAN, DEATHERAGE, and BROWN studied the course of autoxidation of methyl oleate and methyl stearolate at 75° and found the maximum rates of oxidation to be 0·0125 and 0·047 mol oxygen per mole ester per hour.[79] Thus the triple bond compound oxidizes four times as fast as the double bond compound (Fig. 6). Matricaria ester, having two double and two triple bonds conjugated with the carboxyl group, oxidizes without an induction period at a very high initial rate followed by a second phase at a lower rate.[80]

Free acid

The more common unsaturated acids oxidize at maximum rates sometimes twice as great as that of their esters.[17] This effect is probably due to participation of

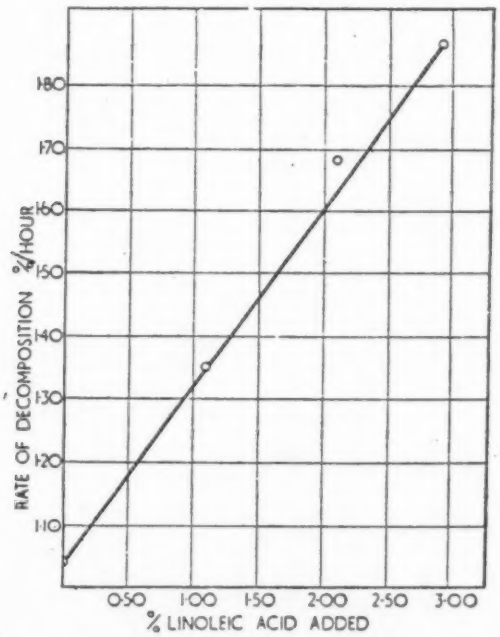


Fig. 9. The effect of the presence of free linoleic acid upon the decomposition of methyl linoleate hydroperoxide at 80°C (from PRIVETT *et al.*).[67]

the carboxyl groups in the decomposition of peroxides. This relationship is indicated by the work of PRIVETT, NICKELL, and LUNDBERG in which addition

of free linoleic acid to methyl linoleate peroxide accelerated its decomposition[64] (Fig. 9). Total oxygen uptake for free oleic acid has also been found to be less than for its esters.[79], [83]

Dilution

According to the law of mass action, dilution of reactants with inert substances would reduce the rate of reaction. This is true in the case of autoxidation of unsaturated oils. The rate of oxidation is roughly proportional to the iodine value of an oil. It is common knowledge that highly unsaturated oils present greater problems of protection against rancidity than do solid, less unsaturated fats.

The effect of dilution of ethyl linoleate upon the rate of linoleate oxidation has been studied by BOLLAND,[51], [57] using both ethyl stearate and ethyl oleate as

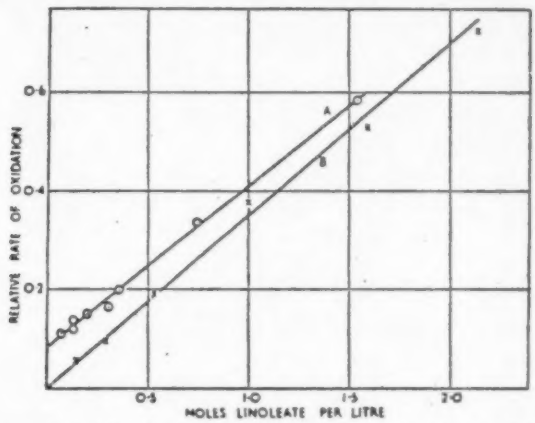


Fig. 10. Influence of ethyl linoleate concentration on rate of oxidation of diluted mixtures at 55°C. (*A*) Ethyl linoleate-methyl oleate. (*B*) Ethyl linoleate-ethyl stearate. Curves *a* and *b* pass through the point for pure linoleate (from BOLLAND).[57]

diluents. When oxygen is not limiting, the relative rate of oxidation varies linearly with linoleate concentration. These data are shown in Fig. 10. This linearity is also shown between oleic acid concentration and its rate of oxidation.[84]

The effect of dilution of fatty materials in solvents is likewise to decrease the chance of collision of free radicals and oxygen with the unoxidized fatty substances. Thus in handling unsaturated materials, danger of oxidation is less in solution than it is in the pure condition. However, when dilution is so high that the concentrations of unsaturated material and dissolved oxygen are of the same magnitude, the proportion of compound which becomes oxidized becomes very high. This has been shown by GROOT and COLPA[85] in the case of vitamin A.

Oxygen pressure

HENDERSON and YOUNG[84] studied the kinetics of oleic acid oxidation and arrived at the rate law expressed in the equation:

$$\frac{-\,dO_2}{dt} = k_1 + k_2\,(\text{peroxide})\,(O_2)^{\frac{1}{2}}$$

Oxygen pressure variation had little effect, however, upon the length of the induction period. BOLLAND'S studies of the kinetics of oxidation of ethyl linoleate led him to formulate the rate equation for autocatalyzed ethyl linoleate oxidation:

$$\frac{-\,dO_2}{dt} = k_2\,(\text{peroxide})\,(\text{linoleate})\,\frac{p}{n_a + p}$$

where p is oxygen pressure and k_2 and n_a are constants. By way of illustration of the effect of oxygen pressure on rate of oxidation of linoleate, an experiment by BOLLAND is shown in Fig. 11.[51]

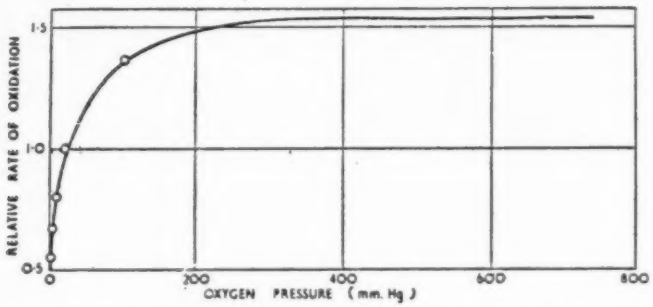


Fig. 11. Effect of oxygen pressure upon rate of oxidation of ethyl linoleate at 45°C (from BOLLAND).[51]

Temperature

It is common knowledge that high temperatures promote rapid autoxidation of fats. This temperature effect is illustrated by the data of PASCHKE and WHEELER[86] in Fig. 12 for peroxidation of soybean oil methyl esters. Similar

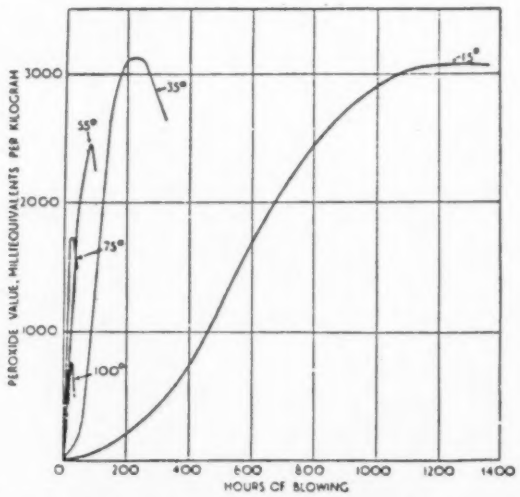


Fig. 12. Peroxide formation in soybean methyl esters autoxidizing at various temperatures (from PASCHKE and WHEELER).[86]

data have been presented by LUNDBERG and CHIPAULT[47] for the oxygen uptake of methyl linoleate. The effects of temperature upon thermal activation of reacting molecules and upon thermal decomposition of peroxides is difficult to separate, but the sum total temperature effect involves these two effects.

The initiation of new chains of reaction is performed by the radicals formed by peroxide decomposition. PASCHKE and WHEELER[86] have demonstrated that rate of peroxide decomposition is dependent upon temperature, and PRIVETT, NICKELL, and LUNDBERG[64] demonstrated this striking effect of

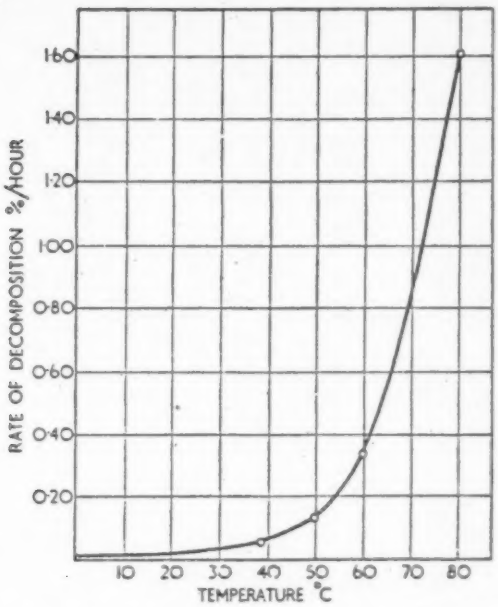


Fig. 13. Effect of temperature upon decomposition of methyl linoleate hydroperoxide (from PRIVETT *et al.*).[87]

temperature upon rate of decomposition of methyl linoleate hydroperoxide (Fig. 13). Near 50°C there is a sharp increase in the rate of decomposition. The ease of decomposition of these peroxides contributes to the difficulty of working with them.

Pro-oxidants

Peroxide catalysis—In the autoxidation of unsaturated substances, the induction period is that initial period of time during which no appreciable oxidation takes place, and during which peroxide does not accumulate rapidly. When peroxide (or partially oxidized material) is added to a fat it has a pro-oxidant action because, in effect, the stage of oxidation is advanced beyond the induction period. Peroxides, being unstable substances, have appreciable thermal decomposition rates, and thus provide free radicals to initiate new chains. The use of benzoyl peroxide is well known for induction of polymerization reactions and has been used as a source of chain-starting radicals in studies

of autoxidation.[55], [57] The effect of benzoyl peroxide upon linoleate oxidation is shown in Fig. 14. Linoleate hydroperoxide has also been used in studies on fat

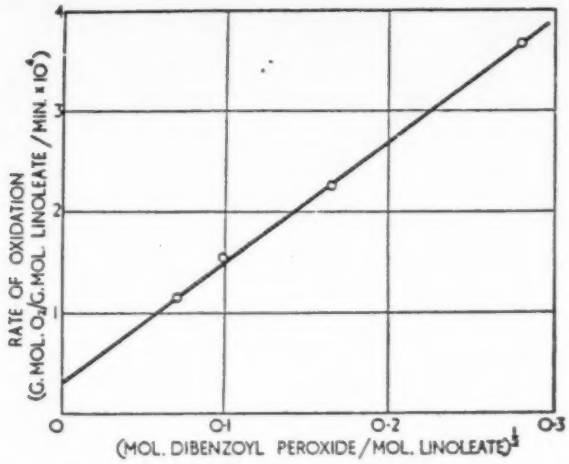


Fig. 14. Effect of benzoyl peroxide upon the oxidation of ethyl linoleate at 100 mm oxygen pressure and 45°C (from Bolland).[51]

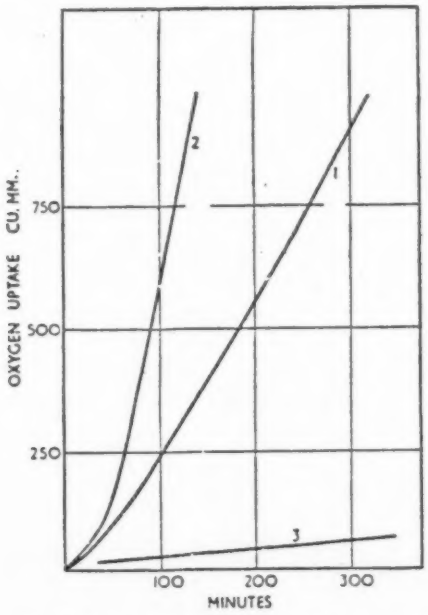


Fig. 15. Effect of metallic driers and antioxidant upon the rate of oxidation of linoleic acid at 60°C in aqueous emulsion. (1) Linoleic acid. (2) Linoleic acid + 5 p.p.m. Cu. (3) Linoleic acid + 0·15 m *M* hydroquinone (from Smith and Stotz).[58]

oxidation, and now that means are available for concentrating them on a large scale by crystallization or urea complex fractionation (see chapter by Schlenk), fat peroxides may find use as polymerization catalysts.

Metal catalysis—In the drying oil industry, pro-oxidants called "driers" are often used to promote rapid oxidation. These substances are salts of heavy metals with organic acids. The effect of driers upon drying time of linseed oil has been extensively studied by LUND[87] and the action of copper upon the oxidation of linoleic acid has been studied by SMITH and STOTZ[88] (Fig. 15). SHELLOX[89] studied the action of various metals upon oleate oxidation and found that lead, aluminium and barium are good catalysts in the primary stage of oxidation, but that zinc is a good catalyst for formation of ketone from peroxide.

The metallic catalysts have their action primarily through the decomposition of peroxides to form additional free radicals. Although to the author's knowledge, no work in the field of fats has treated this phenomenon, it is generally

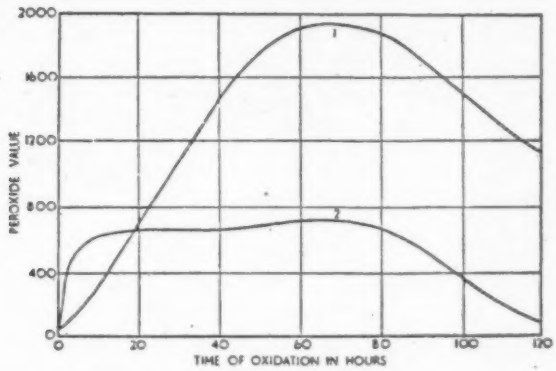


Fig. 16. Effect of cobalt naphthenate drier on the peroxide value of unconjugated and conjugated linoleic acid oxidized at 30°. (1) Linoleic acid. (2) Linoleic acid + drier (from JACKSON and KUMMEROW).[73]

accepted that peroxide decomposition via metallic salts to yield free radicals promotes polymerization[90] and oxidation.[91] However, it is clear from the study by JACKSON and KUMMEROW[73] that in the presence of drier, the peroxide value of oxidizing linoleic acid is held to a lower level (Fig. 16).

Many organic peroxides are able to take electrons from, and yield electrons to metallic cations, depending upon oxidation reduction potential:

$$Cu^{+} + ROOH \rightarrow Cu^{++} + R{-}O{:}^{-} + {\cdot}OH$$

$$Cu^{++} + ROO{:}^{-} \rightarrow R{-}OO{\cdot} + Cu^{+}$$

The metallic catalysts function through maintaining a steady concentration of active hydroxyl radicals.

ANDERSON[92] presents a slightly different view of the metal catalyzed oxidation of methyl linoleate. In his work he found that after the induction period the rate increases to a constant value (r) and can be related to drier concentration (c) by the following relation:

$$r^2 + \alpha r = \beta c,$$

in which α and β are constants dependent upon linoleate concentration. The induction period increases with drier concentration, suggesting that it is related to the oxidation of the cations to a higher valence. Ninety per cent of the absorbed oxygen was found as peroxide. His conception of the mechanism of catalyzed and uncatalyzed autoxidation is the same except that the chain carrier is an addition product of the catalyst rather than the peroxide radical ROO·.

Surface catalysis—The influence of the nature of the vessel upon induction periods and rates of oxidation has been known for some time. The more common surface catalysis is due to contact with atoms of the transition metallic elements in the vessel, and is thus ordinary metal catalysis. However, GEORGE[92] has made a systematic study of surface catalysis by means of the addition to the test sample of inert powders which contained subanalytical amounts of transition element impurities. In common with metal catalyzed and benzoyl peroxide catalyzed oxidations, the surface catalyzed oxidation of tetralin yielded hydroperoxide as the primary product. The surface catalysis is responsible for initiation and termination of reaction chains. See section on physical state of substrates.

Antioxidants

Antioxidants are substances which, when present in small quantities in oils, are able to prevent or delay the oxidation of the oil. They are present in most natural fats and oils, and contribute to the natural stability of raw oils which is often removed by purification. The literature on antioxidant theory and practice has been covered by LUNDBERG up to 1947.[94] Since that time, much of the knowledge of the field has been covered in the Transactions of the Conferences on Biological Antioxidants sponsored by the Josiah Macy Jr. Foundation of New York.

The mechanism of inhibition catalysis was recognized by ALYEA and BÄCKSTRÖM[95] to be by breaking of reaction chains and involving the oxidation of the inhibitor. BOLLAND and TEN HAVE[96] studied the kinetics of ethyl linoleate oxidation in the presence of hydroquinone and came to the conclusion that the inhibitor terminates chains by interaction with peroxide radicals.

$$R—OO\cdot + Hq \rightarrow \text{stable product}$$

From kinetic evidence they also concluded that hydroquinone underwent chemical change. The strong yellow colour of the oxidized mixture suggested that the product of hydroquinone oxidation was benzoquinone. GOLUMBIC[97] found that tocopheral was rapidly oxidized during the induction period of fat oxidation, and when tocopherol had disappeared, the induction period came to an end. LUNDBERG, DOCKSTADER, and HALVORSON[98] studied the kinetics of oxidation of hydroquinone, catechol, nordihydroquaiaretic acid, and gallic acid in oxidizing lard. They found that in each case, antioxidant concentration diminished during initial stages of oxidation and that peroxide did not reach

high values until most of the antioxidant had disappeared. Fig. 17 illustrates the effect.

TAYLOR[99] and MICHAELIS[100] have discussed the mechanism of antioxidant action. MICHAELIS explained antioxidant phenomena on the basis of compulsory univalent oxidation-reduction. Aside from synthetic sulphur compounds, two types of natural antioxidants exist, one which can be reversibly oxidized to quinone, and tocopherol which cannot. They do have the common property of being reversibly oxidized to a semiquinone radical. Semiquinones are well known, and the semiquinone of tocopherol has been demonstrated to exist by MICHAELIS. Tocopherol in alcohol, ether and pentane was cooled to a glassy

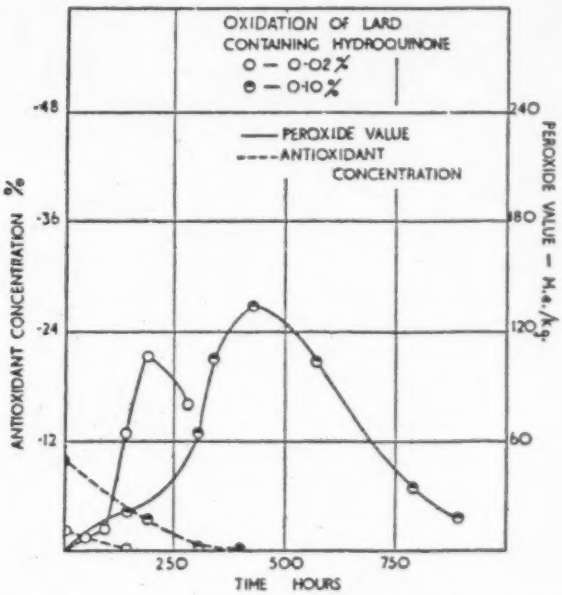


Fig. 17. Oxidation of lard containing hydroquinone as antioxidant (from LUNDBERG *et al.*).[98]

noncrystalline mass. When this rigid solution was irradiated with ultraviolet light, an intense red colour was produced, which disappeared when the glassy solution melted, allowing dismutation of the free radicals. From these observations the most plausible mechanism of antioxidant activity is the following:

$$ROO\cdot + AH_2 \rightarrow ROOH + AH\cdot \qquad \text{primary attack}$$

$$2AH\cdot \rightarrow AH_2 + A \qquad \text{dismutation}$$

or

$$ROO\cdot + AH\cdot \rightarrow ROOH + A \qquad \text{secondary attack}$$

Synergists are substances which reinforce the effect of antioxidants. The synergists may or may not possess antioxidant activities of their own. Synergists are usually dibasic or polybasic organic or inorganic acids. The synergists thus far

receiving the most study are ascorbic acid, phosphoric acid, and citric acid. GOLUMBIC[101] took the view that the function of a synergist was the continual regeneration of the antioxidant at the expense of the synergist which acted as a source of hydrogen.

In a current paper PRIVETT and QUACKENBUSH[102] point out that three factors are incompatible with that mechanism: (1) Some potent synergist combinations do not form oxidation-reduction systems; (2) In the tocopherol-ascorbic acid synergism, no evidence has been presented to define the rate of ascorbic acid destruction; (3) Phosphoric acid has been shown to react with quinone in the absence of hydrogen donors to give a false positive test for tocopherol in the iron-bipyridyl reaction. PRIVETT and QUACKENBUSH found that citric and ascorbic acids suppress the initial accumulation of peroxides which takes place at "pro-oxidant" levels of NDGA and tocopherol in autoxidizing lard. It was also found that ascorbic acid and tocopherol exerted sparing actions on each other in oxidizing lard—the tocopherol was not spared at the expense of ascorbic acid.

The work of PRIVETT and QUACKENBUSH indicates that synergists suppress the "pro-oxidant" action of phenolic antioxidants. This "pro-oxidant" action is a catalysis of peroxide decomposition stimulated by the antioxidant, particularly when it is in high concentration. In an autoxidizing fat containing both antioxidant and synergist, the antioxidant has two actions. The antioxidant terminates oxidation chains by reacting with peroxide radicals:

$$ROO\cdot + AH_2 \rightarrow AH\cdot + ROOH$$

The inhibitor also catalyzes the decomposition of the peroxides, the extent of which is dependent upon antioxidant concentration.

$$ROOH \xrightarrow{AH_2} R\cdot + \cdot OOH$$

The function of the synergist is probably to suppress the antioxidant's catalysis of peroxide decomposition. By suppressing the catalysis, additional chain formation is prevented, and thus the antioxidant molecules are spared from their function in stopping such chains.

Refining procedures

MITCHELL and KRAYBILL,[103] and subsequently BEADLE,[104] have made a study of the effect of refining and bleaching upon the spectral characteristics of vegetable oils. They found that alkali refining lowered the ultraviolet light absorption, and that bleaching with fullers earth raised the absorption if the oil had been oxidized prior to bleaching (Fig. 18). Deodorization likewise caused an increase in absorption at 2700 Å. They demonstrated that bleaching induced conjugation in oils, the conjugation having one more double bond in its system than the original fatty acids contained. The mechanism for this induced unsaturation was suggested to be by oxidation and subsequent dehydration. According to modern oxidation theory this phenomenon could be explained as

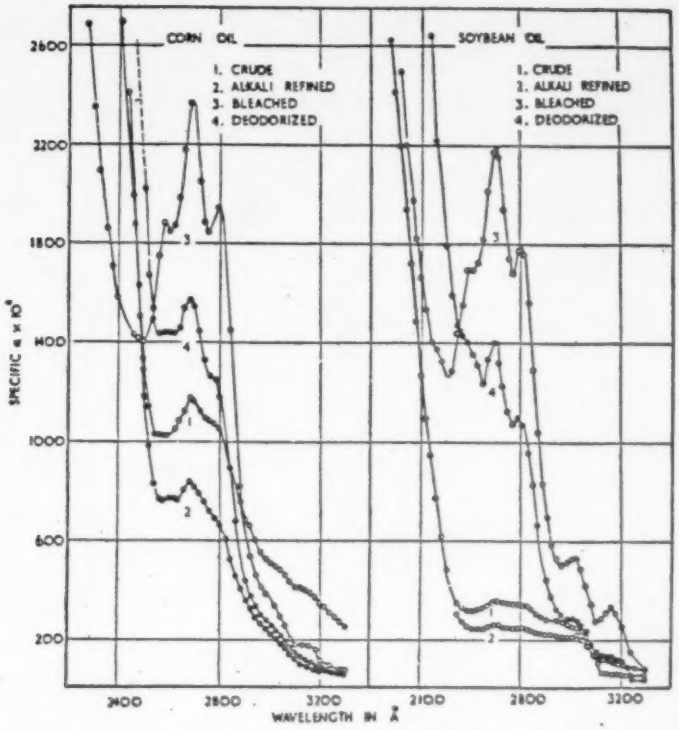
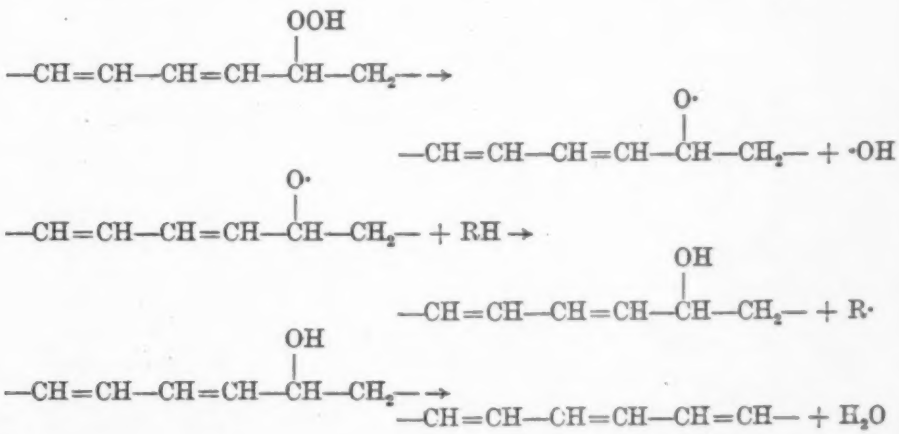


Fig. 18. Effect of processing upon the spectral characteristics of oils (from MITCHELL and KRAYBILL).[103]

follows. Peroxide decomposition could yield a hydroxy radical and a substituted hydroxy radical.



Treatment of oxidized oils with aluminium or magnesium silicate reduces the peroxide value. This is also true for treatment with activated carbon. TÄUFEL

and NAGLI[105] report that treatment with the former decolorizes, delays oxidation, but that treatment with carbon hasten oxidation. It has also been reported that alumina removes antioxidants from oils, thereby reducing their keeping quality.

Physical state of substrates

The degree of dispersion of unsaturated substrates has a marked effect upon their oxidation. The spreading of oil films on porous material can, and often does, lead to spontaneous combustion of the oil. HONN, BEZMAN, and DAUBERT[106], have studied the oxidation of drying oils adsorbed on the surface of finely

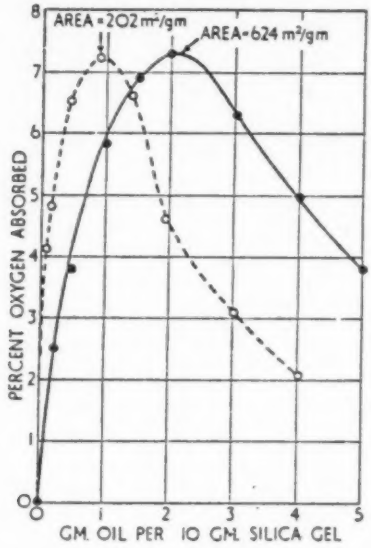
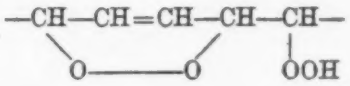


Fig. 19. The effect of oil/solid ratio upon rate of oxidation of soybean oil adsorbed on silica gel (from HONN *et al.*).[106]

divided silica gels. There is a critical oil/solid ratio which yields a maximum rate of oxygen uptake (Fig. 19). The results were interpreted as indicating the existence of a closely packed mono-molecular layer of oil on the adsorbent at the critical ratio, and that this arrangement is most favourable for promoting oxidation. At oil concentrations below the critical oil/solid ratio, the oil molecules are separated by distances dependent upon the ratio, and thus the rate of oxidation depends upon the average distance between oil molecules. Above the critical ratio, the oil molecules form multimolecular films, and the rate of oxidation is decreased, because diffusion of oxygen becomes a limiting factor. This interpretation was verified by the observation that at the critical ratio, the calculated area occupied by that amount of oil as a monolayer nearly equalled the area available on the silica gel surface.

In aqueous colloidal solution, sodium linoleate oxidation appears to be slightly different from oxidation of linoleate esters *en mass*. BERGSTRÖM, BLOMSTRAND, and LAURELL[107] found that in this system the rate of oxidation was dependent

upon copper ions, that maximum oxygen uptake is 2 mol oxygen per mole linoleate, and that the spectral changes were similar to those observed in oxidizing linoleate esters. Isolation of the reaction products as a low viscosity oil indicated that polymerization had been inhibited, and the sharp termination of the reaction at 2 mol oxygen per mole linoleate suggests that the product of oxidation may be rather reproducible. The authors suggested that monomeric diperoxides of the structure



may be formed by the oxidation of the primary peroxide. This method should prove useful in preparation of the product of linoleate oxidation which has 2 mol of oxygen per molecule.

Emulsified oils are subject to oxidation, but because of the instability and non-reproducibility of emulsions, little work has been done on fat oxidation in this type of system, with the exception of enzymatic studies (*vide infra*). However, emulsions are of biological and medical interest. Oil in the dispersed phase is subject to the same type of oxidation as oil in bulk, but the presence of water soluble catalysts has an influence upon the oxidation. Metallic salts and haemoproteins are of particular importance as catalysts in oxidation of biological systems. Much of the catalysis in animal tissue fats which has been considered enzymatic is due to oxidation catalyzed by haemoglobin, myoglobin and catalase.[108]

Most work on fat oxidation has used liquid systems. Oxidation of solid fats is much inhibited by the presence of considerable amounts of saturated fatty acids which act as diluents. On the other hand, oxidation is sharply limited by the solid state of matter in which penetration and diffusion of oxidation is much reduced.

Restriction of unsaturated fatty acids and esters within the crystal structure of urea complexes is effective in inhibiting oxidation of these readily oxidizable substances. The inhibition of oxidation in this case could be either by protection against oxygen penetration, or by prohibition of chain reaction as a consequence of the rigid lattice to which the substrate is restricted. For details the reader is referred to the chapter by SCHLENK in this volume.

Oxidation in the gaseous phase is extremely rapid, but little is known of its mechanism. Contact of hot vapours of fatty acids or esters with air can lead to explosive oxidation.

Irradiation

The oxidation of unsaturated acids and esters is stimulated by various types of irradiation. The absorbed radiant energy activates substrate molecules to the energy level necessary for chemical reaction to take place. Infra-red and visible radiation are somewhat effective, but ultraviolet light, because of its higher energy content, is far more effective. X-ray has recently been found to be effective in inducing fat oxidation.

Ultraviolet light has been used successfully by FARMER and SUTTON[25] to promote the oxidation of methyl oleate to the hydroperoxide. SUTTON[27] used the same method for oxidation of methyl elaidate to the hydroperoxide. SWIFT, DOLLEAR, and O'CONNOR[26] were able to oxidize methyl oleate rapidly by ultraviolet light irradiation and to prepare the hydroperoxide in 90% purity.

BATEMAN and GEE[59] have made a thorough kinetic study of the photo-oxidation process, using cyclohexene, 1-methyl cyclohexene, 2,6-dimethyl-2,6-octadiene, and ethyl linoleate as substrates. They concluded that photo-oxidation proceeds by a chain mechanism in which the generation of free radicals by light absorption is the chain initiation mechanism. The predominant initiation process is the photolysis of the hydroperoxides. When light intensity is fixed and wavelength chosen such that light absorption is weak, the photo-oxidation is autocatalytic, because the products formed are more strongly light-absorbing than the substrate, and their photolysis leads to additional chain formation.

Photo-oxidation is somewhat modified by the presence of chlorophyll. KHAN, LUNDBERG, TOLBERG, and WHEELER[109] have studied the chlorophyll-photo-oxidation of methyl oleate and methyl linoleate. The visible light energy absorbed by the chlorophyll is in some manner transferred to the substrate, thereby activiting the substrate to an energy level sufficient for oxidative attack to take place. The products of linoleate oxidation have a lower light absorption in the ultraviolet region characteristic of conjugated dienes, whereas the products isolated from thermal and plain photo-oxidation are very similar in light absorption. Infra-red studies of the product suggest the presence of isolated *trans* material in the product, whereas this is not found in products of plain photo-oxidation. Chlorophyll photo-oxidized linoleate yielded a peroxide concentrate of high peroxide value, which after reduction to the alcohol and chromatographic separation yielded a significant fraction which was non-conjugated. These results suggest that the 11-hydroperoxido-octadecadienoate can exist and that by some means, becomes a significant product by the action of chlorophyll in spite of the greater thermodynamic stability of the conjugated isomers.

MEAD[110] has found in his studies on x-irradiation of linoleate that the reaction is also a chain reaction. He has measured the quantum yield and found that with increasing concentration, the ionic yield increases. With increasing cysteine concentration, the ionic yield decreases, indicating protection of linoleate against oxidation by sulphydryl compounds. Presumably the mechanism of x-ray stimulated oxidation of linoleate is the same as in autoxidation. The possibility also exists that radiation initiated chain oxidation of unsaturated fat accompanies radiation injury of animals.

Enzymes

Oxidative rancidity involves largely the poly-unsaturated fatty acids, and thus the only enzymatic fat oxidation that appears to be a factor in preservation of fatty foods is that stimulated by lipoxidase. Lipoxidase is a plant enzyme specific for the oxidation of the essential fatty acids and their esters. The enzyme

has been studied carefully, and for detailed information the reader is referred to recent reviews.[15], [108], [111]

Lipoxidase attacks essential fatty acids, yielding hydroperoxide as a primary product. The enzymatic oxidation of linoleate is accompanied by the complete conjugation of the double bonds. The enzyme is active in the range from the freezing point of the solution to somewhat above room-temperature, although the enzyme is inactivated during its action at the higher temperatures.

The most plausible reaction mechanism for linoleate oxidation by lipoxidase involves probable contact between the enzyme and each molecule of the substrate. TAPPEL *et al.*[112] also found that the enzyme was capable of oxidizing antioxidants in the presence of linoleate without the concomittant oxidation of linoleate itself. These reaction mechanisms are given on page 88.

The oxidation of polyphenolic antioxidants by lipoxidase plus linoleate suggests that linoleate may play a role analogous to a coenzyme or prosthetic group in the oxidation of polyphenols and other substances. The reported lipoxidase oxidation of amino acids and polyphenols may be by such a mechanism. If so, the enzyme may be as important in the oxidation of these secondary substrates as in the direct oxidation of poly-unsaturated fatty acids.

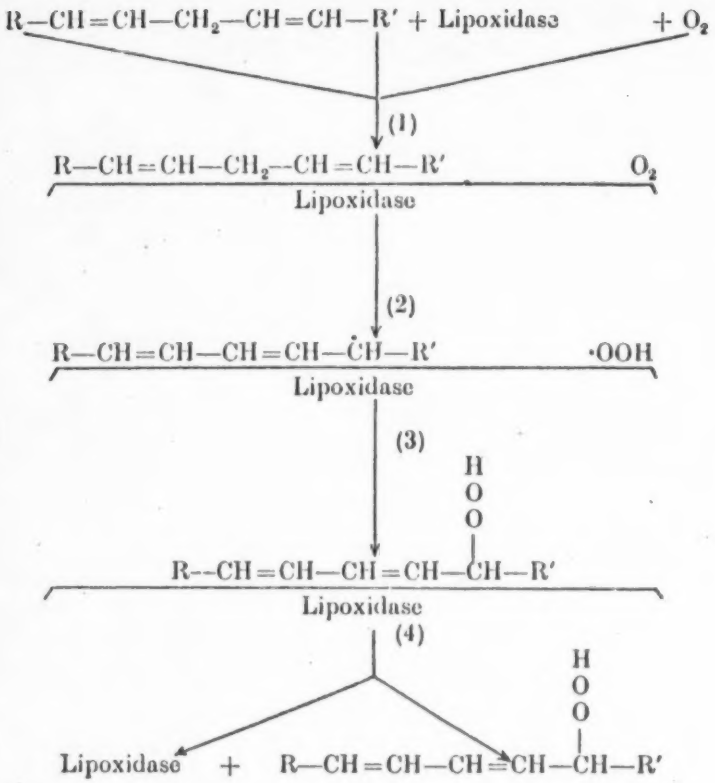
DETECTION OF OXIDATION PRODUCTS

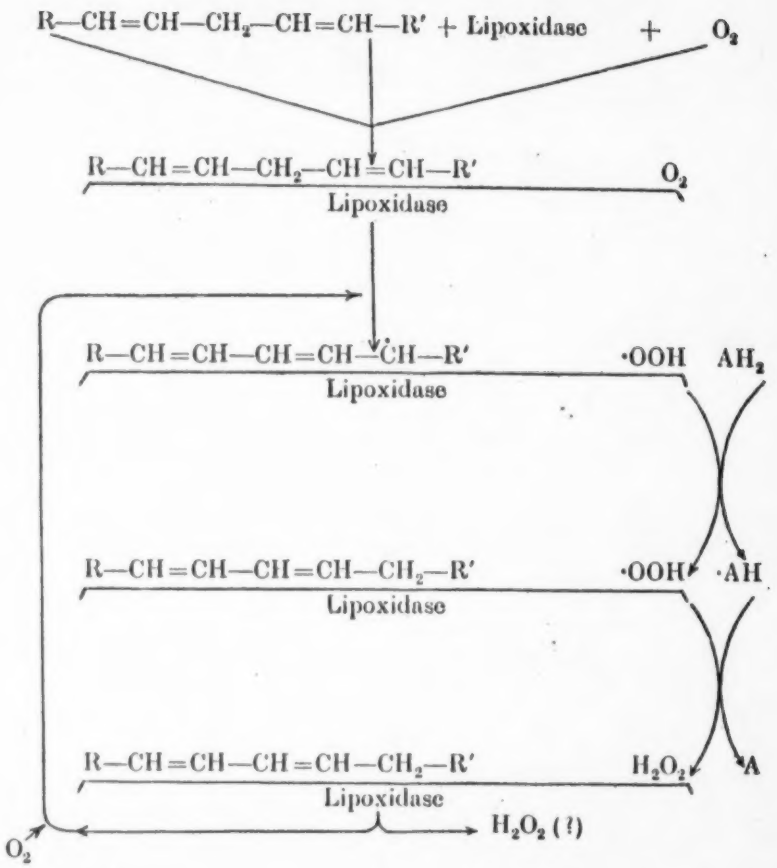
The Kreis test

This test has been used widely for the assay of oxidative status of oils, but it offers the disadvantages of being highly empirical. The test as ordinarily used involves two phase systems or other means of separation of the active components. Modification of the test making it suitable for colorimetry has improved the method.[113] Phloroglucinol has been shown to yield colour with epihydrinaldehyde, malonic dialdehyde, or acrolein treated with H_2O_2,[114] but the presence of these compounds has not been demonstrated in oxidized fat, nor do these substances appear in the currently accepted mechanism of oxidation. Their presence, however, is not unreasonable as secondary oxidation products. Thus the Kreis test, although a proven qualitative test for oxidation, has been of little theoretical value in studies of fat oxidation. It appears that the Kreis test detects substances formed from the decomposition of peroxides, and represents a measure of terminal oxidation products. It may yet prove to be of value in studies of secondary oxidations and peroxide decomposition.

Thiobarbituric acid test

This test has been developed in the past few years, and appears to be related to the Kreis test. It was originally used by KOHN and LIVERSEDGE[115] who observed that animal tissues, upon aerobic incubation, were able to give a colour reaction with thiobarbituric acid. BERNHEIM and his co-workers[116], [117] have traced this reaction to products of oxidation of unsaturated fatty acids, principally linolenic acid. PATTON and KURTZ[118] have studied the reaction involved in the test and applied the test to detection of oxidation in milk fat. They found


R—CH=CH—CH₂—CH=CH—R′ + Lipoxidase + O₂
(1)
R—CH=CH—CH₂—CH=CH—R′ O₂
Lipoxidase
(2)
R—CH=CH—CH=CH—CH—R′ ·OOH
Lipoxidase
(3)
H O O
R—CH=CH—CH=CH—CH—R′
Lipoxidase
(4)
H O O
Lipoxidase + R—CH=CH—CH=CH—CH—R′


R—CH=CH—CH₂—CH=CH—R′ + Lipoxidase + O₂
R—CH=CH—CH₂—CH=CH—R′ O₂
Lipoxidase
R—CH=CH—CH=CH—CH—R′ ·OOH AH₂
Lipoxidase
R—CH=CH—CH=CH—CH₂—R′ ·OOH ·AH
Lipoxidase
R—CH=CH—CH=CH—CH₂—R′ H₂O₂ A
Lipoxidase
H₂O₂ (?)
O₂

that malonic dialdehyde gave a strong test and that the thiobarbituric acid test is much more sensitive than the Kreis test. The latter did not begin to yield measurable colours until after decided oxidized flavours appeared, whereas the thiobarbituric acid test gave measurable responses during the development of perceptible rancidity. The TBA test appears, therefore, to hold more promise than does the Kreis test. It would be of value in detection of oxidation in its early stages.

Stamm test

This test depends upon reaction of oxidized fat with s-diphenyl carbazide to yield a colour.[119] It has been correlated with organoleptic detection of rancidity and found to give a rather good correlation.[120]

Dicarbonyl compounds

These compounds are supposedly present among the products of oxidation of fats and O'Daniel and Parsons have postulated that the colour developed by oxidized fats in alkali is due to the formation of quinones from α-dicarbonyl compounds by double aldol condensation.[121] They suggested that alkali colour is a test for α-dicarbonyls. Prill has also developed a colour test involving their dioximes.[122] The alkali colour test has been shown to be affected by fat concentration and by time.[120] Nevertheless, the α-dicarbonyl test was considered the best method for assessing rancidity because the dicarbonyl value of oils is not affected by heat treatments of the oils.

Alkali colour

When oxidized fats are dissolved in an alkaline medium, intense orange or red colours are produced. This is the result of production of chromophores whose principal absorption is in the ultraviolet range. The visible colour is merely end absorption of ultraviolet chromophores. The alkali colour has been studied spectrophotometrically by Holman *et al.*,[44] and it was concluded that the colour was probably not due to quinones formed from dicarbonyl in the oxidized fat. Jasperson *et al.*[123] also concluded that dicarbonyl tests on oxidized fats are fundamentally different from those on model compounds. Present opinion is that the colour is due to condensation of unsaturated carbonyl compounds. Chipault *et al.*[47] have found that the alkali colour is due to two reactions, one which is instantaneous, and one which continues over long periods.

Hendrickson, Cox, and Konen[50] used spectral study of the alkali colour in assessing the oxidative changes taking place in film formation. Chipault *et al.*[47], [77], [78] likewise used this colour formation in describing the chemical changes during film formation from pure triglycerides, pentaerythritol esters, and alkyds prepared from known pure fatty acids.

It would appear that the α-dicarbonyl colour reactions, based upon sketchy knowledge, however, do possess merit for assessing oxidation of oils. It is to be hoped that a better understanding of their chemistry will lead to more effective use.

Iodometric peroxide value

Peroxide number is the most commonly used assay of state of oxidation in fats and oils. Many methods have been devised for its measurement and BARNARD and HARGRAVE[124] have discussed thoroughly the merits of the various methods.

To date the iodometric methods appear to be most satisfactory and have been the most used. The methods originally devised by LEA[125] and WHEELER[126] have been used widely, but recent studies show that improvement can be made by the exclusion of oxygen from reagents and reaction flask.[127] This method measures the primary product of oxidation, and because peroxides are subject to rapid destruction under some conditions, the peroxide value does not completely describe the oxidative history of an oil.

Ferric thiocyanate method of peroxide determination

This method lends itself to colorimetry, but is subject to considerable error. CHAPMAN and MCFARLANE[128] describe such a method, but the peroxide as determined by this means is far higher than that found by iodometric methods. LEA[129] found that by exclusion of oxygen from the reaction medium the colour formation is diminished to about one-fourth that obtained in the presence of oxygen, and that the values were lower than theoretical. The thiocyanate method has its use in comparative studies where a quick colorimetric method is desired, but it requires rigid exclusion of air for reproducibility. The values obtained in the presence of air are proportional to, but higher than iodometric values. It is more sensitive than the iodometric method and can be used in lower ranges of peroxide value.

Dichlorophenol-indophenol peroxide determination

This method, introduced by HARTMANN and GLAVIND[130] likewise yields high values in the presence of air.[131] However, the values are reproducible and are useful only in comparative studies. Perhaps the most significant use of this reagent has been to detect the presence of peroxides histochemically by GLAVIND *et al.*[132]

Stannous chloride peroxide value

Stannous chloride has been used as reagent for estimation of organic peroxides by several workers with varying degrees of success. BARNARD and HARGRAVE[133] have reviewed the literature on the use of this method of peroxide estimation, and have proposed a modification they believe to be satisfactory. The method is titrimetric and requires about 1 milliequivalent of peroxide for determination. PRIVETT *et al.*[134] use a method which is a modification of the stannous chloride procedure, but which is colorimetric and which requires 0·5 to 25 microequivalents of peroxide. This method holds promise of applicability to low levels of oxidation, and the colorimetric feature lends the method to routine use. The reaction is performed in alcohol in the presence of dichlorophenol-indophenol. After the reduction of the peroxide by stannous chloride, the medium is made acid with oxalic acid, and the stannous chloride remaining then reacts with the dye.

Ultraviolet absorption

Oxidation of poly-unsaturated fatty acids is accompanied by increased ultraviolet absorption. The magnitude of change is not easily related to degree of oxidation because the effects upon the various unsaturated acids are different in quality and magnitude.[45] However, the spectral change for a given substance can be used as a relative measure of oxidation, and probably has its best application in the detection of oxidation rather than its measurement. The examination of ultraviolet spectrum is a rapid and sure method for assessing the purity and freshness of unsaturated fatty materials. The higher the absorption, the greater has been the exposure to oxygen.

Infrared analysis

HENICK[135] has applied infrared analysis to the detection of oxidation products in milk fat. Spectral changes were detected before off flavours developed, and both loss of flavour and development of off flavours were correlated with definite absorption bands. The subject of infrared analysis of fats and oils will be treated by WHEELER in another chapter in this volume, and the reader is referred to his treatment for more information on its application to problems of oxidation.

Polarographic analysis

Polarography has recently been used in the analysis of fat oxidation products. LEWIS, QUACKENBUSH, and DE VRIES[136] found a linear relationship between wave height and peroxide value in the early stages of the oxidation of fats

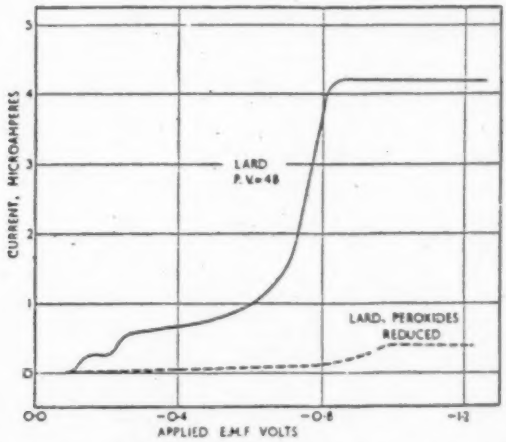


Fig. 20. Example of polarograph curve for oxidized and reduced lard. Lard concentration in 0·3 M lithium chloride was 1% (from LEWIS and QUACKENBUSH).[137]

(Fig. 20). At more advanced stages of oxidation the polarograph does not reduce all substances capable of reduction by acidic iodide solution. They also found that fat peroxides showed a triple wave in the current voltage curves in neutral solution. They were unable to distinguish the peroxides of methyl oleate or

methyl linoleate[137] but found three types of peroxide present. The temperature at which autoxidation took place also affected the type of polarographic curve obtained.

WILLITS *et al.*[138] have studied the polarographic behaviour of forty-one oxygen-containing compounds in a non-aqueous electrolytic medium. They concluded that peroxides, hydro-peroxides, aldehydes, ketones conjugated with double bonds and diketones can be measured polarographically. It appears that polarographic analysis has promise of becoming a powerful tool in the study of fat oxidation.

BIOLOGICAL SIGNIFICANCE OF OXIDIZED FAT

The adverse effect of the ingestion of rancid fat is well known. The subject has been reviewed by BURR and BARNES[139] in 1947 and by QUACKENBUSH in 1945,[140] and they concluded from the evidence at that time that one of the chief deleterious effects of dietary rancid fat is the destruction of vitamins and perhaps other dietary essentials. On the other hand, many symptoms observed are not explained in terms of vitamins, and at least for the present it must be assumed that rancid fat nas a direct toxic effect.

Vitamin destruction

Rancid fat in the diet causes the development of a biotin deficiency because of the oxidation of biotin which is synthesized in the intestine.[141] This is in

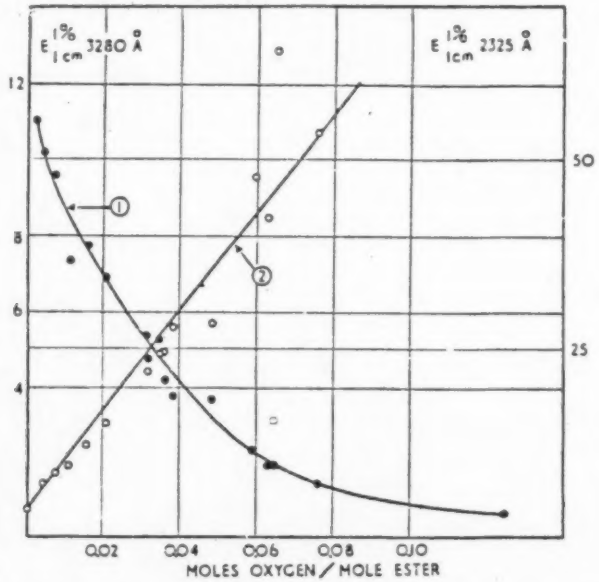


Fig. 21. Loss of vitamin A during autoxidation of vitamin A-methyl linoleate mixtures. (1) Vitamin A, 3280 Å. (2) Diene conjugation 2325 Å (from HOLMAN).[146]

agreement with observations that biotin is inactivated by rancid fats *in vitro*.[142] Ascorbic acid may be partially destroyed as a consequence of its synergistic

antioxidant action;[102] pyridoxine and pantothenic acid have been found to relieve acrodynic rats fed rancid fat.[143] Thus there may be some relationship between the toxicity of rancid fat and these vitamins.

The fat soluble vitamins are subject to oxidation in rancid fats. Tocopherol is easily oxidized, and would occur in very low quantities in rancid diets because it is destroyed before the active rancidification begins. This is likewise true for carotene and vitamin A. These substances are subject to coupled oxidation in oxidizing fat, and are oxidized early in the process of development of rancidity.[144], [145] This is shown in Fig. 21 for vitamin A. Vitamin D is also subject to oxidation under conditions which prevail in dietary preparations.[146]

Essential fatty acid destruction

It is of course obvious that if rancidity is caused largely by the oxidation of poly-unsaturated fatty acids, these essential fatty acids are destroyed in the course of the process. Unfortunately, this aspect of rancid fat toxicity has had little attention until recently. In the study of the ability of rancid fat and oxidized esters to relieve the symptoms of acrodynia—a deficiency disease caused by removal of pyridoxine and essential fatty acids from the diet—oxidized methyl linoleate relieved the symptoms but was not as effective in doing so as fresh ester. Tocopherol did not improve the response to the rancid preparations. Highly oxidized preparations were ineffective.[143]

Recent discussions at the Macy Conferences on Biological Antioxidants suggested that one role of tocopherol *in vivo* may be to provide an antioxidant status for the protection of essential fatty acids (poly-unsaturated fatty acids). To test this hypothesis WITTEN and HOLMAN[147] attempted to simulate pro-oxidant status by feeding benzoyl peroxide with the essential fatty ester supplement, and to simulate antioxidant status by feeding added tocopherol with the supplements. The conversions of linoleate and linolenate to more highly unsaturated fatty acids by fat deficient rats were used as an index of essential fatty acid utilization. It had been previously found that in rats, linoleate induces synthesis of arachidonate and linolenate induces synthesis of hexaenoate, with other cross conversions also taking place.[148] When benzoyl peroxide plus linoleate was fed as a supplement to fat-deficient rats, growth response was greatest, least when benzoyl peroxide was fed alone. Fat synthesis was found to be stimulated by benzoyl peroxide plus unsaturated ester. Benzoyl peroxide plus linoleate led to the formation of hexaenoic acid, but other conversions appeared to be unaffected by either tocopherol or benzoyl peroxide. Thus, contrary to expectation, the pro-oxidant, which is toxic when fed alone, proved to be beneficial according to criteria of growth and fat synthesis.

From the above results it appeared that oxidized essential fatty acids are involved in some of the conversions. To explore this question farther, HOLMAN and GREENBERG[149] have administered to fat deficient rats the following supplements: ethyl linoleate, partially oxidized ethyl linoleate, ethyl linoleate with dietary benzoyl peroxide, ethyl linoleate hydroperoxide, thermally decomposed ethyl linoleate hydroperoxide and conjugated ethyl linoleate. Fresh linoleate,

oxidized linoleate and linoleate plus benzoyl peroxide all relieved the dermal symptoms of fat deficiency, reduced the water consumption of the rats, and stimulated arachidonate synthesis. Linoleate peroxide, decomposed peroxide, and conjugated linoleate were not effective as judged by these criteria. Thus the concentrated products of linoleate oxidation and conjugated linoleate could not be utilized as essential fatty acid. However, fresh linoleate, slightly oxidized linoleate, and linoleate plus dietary benzoyl peroxide were curative. The beneficial effect of feeding the catalyst for autoxidation, benzoyl peroxide, plus linoleate together in supplement suggested that a more oxidizable medium may be better utilized by the animal. The influence of pro-oxidant and antioxidant conditions upon essential fatty acid metabolism is still not clear, and it is obvious that much experimental evidence and verification will be needed before the matter is understood.

It seems clear from these latter studies that the products of fat oxidation are not well tolerated by animals. The oxidation products cause intensification of the symptoms of fat deficiency and appear to be toxic. It is not clear yet whether the toxicity is due to an effect upon intestinal bacterial synthesis of vitamins, or due to a direct toxicity.

Effect of oxidized fat on enzyme systems

BERNHEIM, WILBUR, and KENASTON[150] have recently demonstrated an inhibition of enzyme action by fatty acid oxidation products. Washed tissue suspensions or mitochondria, when incubated with ascorbic acid, lose enzymatic activity. The decrease in succin-oxidase, cytochrome oxidase, and choline oxidase activities has been found to parallel the amount of oxidized unsaturated fatty acid as measured by the thiobarbituric acid test (see p. 87). The enzyme inactivation can be prevented by quercetin, which inhibits oxidation. Oxidized methyl linolenate also inactivates the enzymes. These observations are of interest particularly in the case of cytochrome oxidase, which is known to be associated with lipid containing some highly unsaturated acids. It may well be that the inhibition is due to the oxidative destruction of essential fatty acids which are a part of the active enzyme system.

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3

NUTRITIONAL SIGNIFICANCE OF THE FATS*

Harry J. Deuel, Jr.

I. Introduction

Until recently, many nutrition workers have considered fats as optional components of the diet. It was believed that an equally good nutritional condition obtained in the absence of fat as when this foodstuff was present, provided that the diet contained sufficient calories and was otherwise complete. However, since the classical discovery of Burr and Burr[1] that certain unsaturated fatty acids are essential for growth and, in fact, for survival, it has been more generally recognized that fats may serve as a required nutrient, not only as a source of the essential fatty acids but probably also in a variety of other ways.

A number of important functions of fat are generally recognized. In the first place, fat is the foodstuff which possesses the greatest caloric density of any of the foodstuffs. In the dry state, it yields 9·3 large calories† per gm, as contrasted with values of 4·1 calories for protein and carbohydrate. Moreover, fat is hydrophobic and usually occurs without water, while both protein and carbohydrate are hydrophilic. Therefore, in nature, the ratio of caloric value in foods between fat on the one hand and protein and carbohydrate on the other hand, is usually greater than the 2·3 : 1 ratio which exists between these foodstuffs in the dry state. It is obvious that fat is the foodstuff to be employed in increasing amounts when the bulk of a diet must be controlled.

A second function of fat is as a carrier of the fat-soluble vitamins. Not only does fat act as an excellent solvent for the carotenoids, vitamins A, vitamins D, and the tocopherols, but it also protects them from oxidation to a much greater extent than if they were present in an aqueous suspension. Moreover, the presence of fats in the gastrointestinal tract concomitantly with the fat-soluble vitamins may aid in the absorption of the latter. Phospholipids, which occur to a greater or lesser extent in most natural fats, furnish choline, necessary for the synthesis of methionine, and as a general transmethylating agent. The importance of dietary fat is not confined to the fat-soluble vitamins, but its presence is also vital in some of the water-soluble vitamins. Thus, Evans and Lepkovsky[2] demonstrated in 1929 that fats exhibit a thiamin-sparing action, although it was later reported[3] that this foodstuff did not possess any riboflavin-sparing activity. On the other hand, the pyridoxine requirement has been shown to be dependent upon the amount of fat in the diet.[4], [5]

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† The term, calories, as used in this chapter refers to kilocalories (1000 small calories).

Fats are necessary in the diet to improve the palatability of the food. Low-fat diets prove very unappetizing; under conditions in which such a diet is required, it may bring about a voluntary reduction in food consumption. STARLING[6] stated that the reduced food intake in Great Britain during World War I was definitely associated with the low-fat content of the diet. A considerable number of the population lost weight because of their failure to consume an adequate number of calories to maintain an energy equilibrium on the high-carbohydrate, low-fat diet then available. Baked foods are likewise virtually impossible without fats.

II. Physiological Functions in which Fats play a Role

1. Fats as the Source of Essential Fatty Acids

1. *Discovery of the necessity of essential fatty acids*

The first proof that the exclusion of fats from the diet resulted in a fat-deficiency syndrome was obtained as recently as 1926 by EVANS and BURR.[7] Shortly thereafter, MCAMIS *et al.*[8] likewise noted that rats receiving fat in their diets grew better than did animals on fat-free regimens; however, they failed to report on any deficiency symptoms in the rats receiving the fat-free diet. It remained for BURR and BURR[1] to demonstrate clearly for the first time that this pathological deficiency was a new dietary disease which was not to be ascribed to lack of vitamins A or D.

When young rats are subjected to a fat-free diet, they grow at a normal rate for several weeks. However, after 3 or 4 weeks, the growth curve begins to flatten as compared with that of rats receiving cottonseed oil, until a plateau is reached after 9 to 10 weeks, followed by a moderate decline in weight.[9] When the rats are continued on the fat-free regimen for a prolonged period, their weight may decline further, and premature death occurs. BURR[10] has listed the typical symptoms in the rat as follows: (1) marked retardation in growth; (2) development of scaly skin and caudal necrosis; (3) kidney lesions with concomitant haematuria; and (4) death. Kidney lesions were found in 100% of the rats examined at autopsy, while the incidence of caudal necrosis was variable. Other less specific lesions which were noted include histological changes in the ovaries, uterus, and other tissues, resulting in poor ovulation, impaired reproduction and lactation in the female, and complete sterility in the male. Deficient rats likewise exhibit histological abnormalities of the epidermis. WILLIAMSON[11] has shown that the epidermis of rats on fat-free diets becomes thicker and more differentiated than that of normal rats. The stratum granulosum becomes especially distinct, and the horny layer thick. This syndrome can be entirely prevented when essential fatty acids are given. Another symptom noted by BURR was an excessive water intake. A high metabolic rate also obtains, associated with a high respiratory quotient.

BURR and BURR[12] reported somewhat later that the fat-deficiency symptoms disappeared immediately, and that a dramatic recovery from the deficiency

condition occurred when lard, cod-liver oil, or liver was fed. It was shown that the curative agents were such unsaturated acids as linoleic, linolenic and arachidonic acids. It was suggested that the varying potencies of different fats in curing the fat-deficiency symptoms might be correlated with the proportion of the component "essential" fatty acids in the particular fat under consideration.

2. *Compounds active as essential fatty acids*

The curative effects on the so-called fat deficiency disease could be traced to specific fatty acids present in the neutral fats. This was evident immediately after the first recognition of the deficiency. The glycerol moiety was shown to be completely without prophylactic action.[1] Saturated fatty acids such as stearic, palmitic, myristic, lauric, and lower acids were likewise found to be inactive, as was evident from the negative results obtained with coconut oil, with hydrogenated coconut oil, and with methyl stearate.[12] In fact, EVANS and LEPKOVSKY[13] and SINCLAIR[14] have reported that the feeding of hydrogenated coconut oil to rats on an otherwise fat-deficient diet does not alleviate the fat-deficiency symptoms, but in fact actually accentuates them. As a result, the essential fatty acid requirement for growth is increased by the concomitant ingestion of such saturated fats. DEUEL and co-workers[15] have recently confirmed the earlier findings as regards the deleterious effect of hydrogenated coconut oil when administered to rats suffering from fat-deficiency symptoms. SINCLAIR[16] has reported that diets containing a considerable proportion of elaidin accentuate the essential fatty acid deficiency in the same way as does hydrogenated coconut oil.

Although oleic acid was at first thought to have some slight activity as a therapeutic agent,[12] BURR, BURR, and MULLER[17] later showed it to be completely inactive in this respect. The fact that the saturated fatty acids cannot cure the fat-deficiency syndrome is entirely reasonable, as deduced from our knowledge of intermediary metabolism, since the diet employed on which the deficiency develops contains both carbohydrate and protein. Both of these foodstuffs are readily convertible to fats having such saturated fatty acids. This fact has been repeatedly demonstrated over recent years.[18] Since unsaturated acids such as oleic acid may normally be derived from the saturated acids,[19] it is likewise obvious that oleic acid could not be expected to exert a curative effect. The fatty acids which prevent or cure the fat-deficiency symptoms are therefore compounds which cannot be synthesized *de novo* in the body, and which must be obtained from the diet.

The most effective unsaturated fatty acids which are classed with these so-called "essential" fatty acids are: the dienoic acid, linoleic acid,[10], [17] 9,12-octadecadienoic acid, the trienoic acid, linolenic acid,[17] 9,12,15-octadecatrienoic acid, and the naturally-occurring tetraenoic acid, arachidonic acid,[20], [23] 5,8,11,14-eicosatetraenoic acid. A list of the unsaturated acids which have been tested for curative action in fat-deficiency disease is given in Table 1.

Table 1. Summary table listing the purified fatty acids which have been tested for activity in curing fat deficiency symptoms

Common name	Systematic name and formula	Reference
	Positive response	
Linoleic acid	9,12-Octadecadienoic acid $CH_3(CH_2)_4CH : CHCH_2CH : CH(CH_2)_7COOH$	10
Linoleyl alcohol	9,12-Octadecadienol $CH_3(CH_2)_4CH : CHCH_2CH : CH(CH_2)_7CH_2OH$	23, 24
Linolenic acid	9,12,15-Octadecatrienoic acid $CH_3CH_2CH : CHCH_2CH : CHCH_2CH : CH(CH_2)_7COOH$	17, 25–28
Arachidonic acid	5,8,11,14-Eicosatetraenoic acid $CH_3(CH_2)_4CH : CHCH_2CH : CHCH_2CH : CHCH_2CH : CH(CH_2)_3COOH$	20–23, 29
†	Docosahexaenoic acid Formula in doubt	30
Hexahydroxystearic acids (linusic and isolinusic)	9,10,12,13,15,16-Hexahydroxyoctadecanoic acid $CH_3CH_2(CHOH)_2CH_2(CHOH)_2CH_2(CHOH)_2(CH_2)_7COOH$	30
	Negative response	
Oleic acid	*cis*-9-Octadecenoic acid $CH_3(CH_2)_7CH : CH(CH_2)_7COOH$	17, 20, 31
Elaidic acid	*trans*-9-Octadecenoic acid $CH_3(CH_2)_7CH : CH(CH_2)_7COOH$	16, 27
12-Oleic acid	12-Octadecenoic acid $CH_3(CH_2)_4CH : CH(CH_2)_{10}COOH$	23
Erucic acid	13-Docosenoic acid $CH_3(CH_2)_7CH : CH(CH_2)_{11}COOH$	23
Ricinoleic acid	12-Hydroxy-9-octadecenoic acid $CH_3(CH_2)_4CHOHCH_2CH : CH(CH_2)_7COOH$	23

Linolelaidic acid	*trans*-9-*trans*-12-Octadecadienoic acid $CH_3(CH_2)_4CH : CHCH_2CH : CH(CH_2)_7COOH$	33
9,11-Linoleic acid	9,11-Octadecadienoic acid $CH_3(CH_2)_5CH : CHCH : CH(CH_2)_7COOH$	33
α-Elaeostearic acid	9,11,13-Octadecatrienoic acid $CH_3(CH_2)_3CH : CHCH : CHCH : CH(CH_2)_7COOH$	17
Dioxidostearic acid	9,12-Dioxidooctadecanoic acid $CH_3(CH_2)_4CH—CHCH_2CH—CH(CH_2)_7COOH$ (each CH—CH pair bridged by O)	30
Trihydroxystearic acid*	9,10,12-Trihydroxyoctadecanoic acid $CH_3(CH_2)_5CHOHCH_2CHOHCHOH(CH_2)_7COOH$	30
Tetrahydroxystearic acid	9,10,12,13-Tetrahydroxyoctadecanoic acid $CH_3(CH_2)_4CHOHCHOHCH_2CHOHCHOH(CH_2)_7COOH$	30
Chaulmoogric acid	13-(2-Cyclopentenyl)-*n*-tridecanoic acid $CH_2—CH_2—CH \cdot (CH_2)_{12}COOH$ $CH=\!=\!=CH$ (ring closed to the CH_2 and CH above)	23, 30
†	4,8,12,15,19-Docosapentaenoic acid $CH_3CH_2CH : CH(CH_2)_2CH : CHCH_2CH : CH(CH_2)_2CH : CH(CH_2)_2CH : CH(CH_2)_2COOH$	26
†	2-Phytenic acid $(CH_3)_2CH(CH_2)_3CH(CH_2)_3CH(CH_2)_3C : CHCOOH$ (with CH_3 on each of the three branch carbons)	33
†	2,6-Phytadienoic acid $(CH_3)_2CH(CH_2)_3CH(CH_2)_3C : CH(CH_2)_2C : CHCOOH$ (with CH_3 on each of the three branch carbons)	33
†	10,13-Nonadecadienoic acid $CH_3(CH_2)_4CH : CHCH_2CH : CH(CH_2)_8COOH$	33
†	11,14-Eicosadienoic acid $CH_3(CH_2)_4CH : CHCH_2CH : CH(CH_2)_9COOH$	33

* Includes α, β, γ, and δ-acids. † No common name for these acids.

3. *Comparative biopotency of essential fatty acids*

a. Requirement for linoleate

Most of the studies on the curative effects of the essential acids have been made with linoleic acid, but there is no general agreement as to the optimum dosage required by rats. According to BURR,[10] no absolute values can be given for the amount of linoleate which should be considered as optimum. It has been repeatedly shown that amounts of linoleate as low as 5 mg will produce definite growth when tested with rats on the fat-deficient diets, while BURR *et al.*[20] and HUME and associates[21] reported marked response in body weight gains when doses of 10 to 20 mg of the essential acid were fed. BURR and collaborators[20] cited a figure of 40 mg as the optimum dosage, while HUME *et al.* have recorded 42 mg[21] as the linoleate requirement. On the other hand, MARTIN[25] states that 30 mg of linoleate, or slightly less, is the most satisfactory level, and this conclusion has been supported by MACKENZIE and co-workers.[34]

However, other workers reported augmented growth when the linoleate intake was considerably higher, as compared with that at lower dosages. TURPEINEN[24] found that 100 mg was the optimum value for linoleate in female rats.

More recent experiments of the DEUEL group have placed the figure for linoleate requirement even higher for male rats. Although GREENBERG and associates[28] originally postulated, on the basis of a straight line curve when log dose and body weight gain were plotted, that the optimum requirement for male rats was at least 100 mg daily, later results indicated that the figure exceeded 100 mg.[29] Ultimately it was shown that the maximum growth level was not attained even when 200 mg dosages were administered.[15] It was also found that the daily doses of 400 mg of methyl linoleate were toxic, although the possibility exists that the toxicity might be related to the high intake of methyl groups. Further experiments are necessary to establish the optimum figure for linoleate in male rats.

(*a*) *Sex difference in requirement*—One interesting development which was reported by GREENBERG *et al.*[29] was the demonstration of a sex difference in the requirement for essential acids. Although the optimum level for male rats exceeds 200 mg per day,[15] that for females is only a fraction of this value. According to earlier work,[28] the requirement based on growth response appeared to be in the neighbourhood of 10 to 20 mg for females; in later experiments, the highest growth was obtained on a 50 mg dosage, while growth was actually depressed when the linoleate intake was increased to 100 mg per day. The differences in essential fatty acid requirement between the male and female rats were later shown to be independent of the α-tocopherol requirement.[35]

b. Requirement for linolenate

Although the term, "essential fatty acids" is generally used, BURR[10] considers that any one of the acids alone may serve to cure the deficiency symptoms. BURR and associates[17] reported that the growth-promoting effects of linoleic and linolenic acids were additive. MARTIN,[25] however, disagrees with this view.

It was later found that linolenic acid appeared to be more active than linoleic acid in its anti-dermatitis action, and equal to it in its growth-promoting activity.[20]

In recent experiments of GREENBERG *et al.*,[28] linolenic acid, fed alone, was found to be markedly inferior to linoleic acid, both as a growth-promoting agent and in its curative effect on the dermatitis of fat-deficiency disease. Thus, no appreciable growth response was noted when the daily dosage of linolenate was 10 mg, although the same dosage of linoleate gave a marked response. However, when as small an amount as 10 mg of linoleate was administered to the rat on the fat-deficient diet, together with 10 mg of linolenate, the growth response was as great as that to 20 mg of linoleate alone. GREENBERG *et al.*[28] postulate that a "sparking action on the part of linoleate is required before linolenate can play its role as an essential acid."

c. Requirement for arachidonate

Although arachidonic acid is not a normal component in vegetable fats, it is considered by TURPEINEN[23] and SMEDLEY-MACLEAN and NUNN[22] that it is the principal unsaturated fatty acid required by the animal organism. As the result of a number of independent investigations by BARKI *et al.*,[36] by NUNN and SMEDLEY-MACLEAN,[37] by REISER,[38], [39] and by RIECKEHOFF and co-workers,[40] it has been established that both linoleic and linolenic acids undergo an interconversion in the animal organism to give rise to arachidonic acid. WIDMER and HOLMAN[41] reported that, when fat-deficient rats were given linoleate supplements for two months, increased levels of arachidonate were found in the heart and liver. In negative control rats which received stearate or oleate, no increase in polyunsaturated acids in these organs could be noted.*

If arachidonic acid is the ultimate unsaturated acid required by the animal organism, one would expect it to exhibit the highest biopotency of any of the essential fatty acids. Although the experimental results have been somewhat divergent in demonstrating such a superiority, the bulk of evidence would seem to support the viewpoint that arachidonic acid is superior to other unsaturated acids in supplying the need for essential fatty acids.

TURPEINEN,[23] in 1938, reported that arachidonic acid possesses three times the biopotency of linoleic acid when tested by the gain-in-weight technique. Using the same index of activity, HUME and co-workers[21] found the ratio of biopotencies of methyl arachidonate and methyl linoleate to be 2 : 1. On the other hand, in the earlier experiments of BURR, BURR, and MILLER[17] an inferior growth-promoting activity was actually noted for methyl arachidonate when fed with a mixture of methyl linoleate and methyl linolenate. These workers later indicated that the methyl arachidonate sample was one of poor quality.

* *Editor's Note:* The metabolic interconversions of the essential fatty acids and the factors affecting them are subjects outside the scope of the present discussion. The reader will find these discussed in the following papers: (1) *Proc. Third Conf. Research Amer. Meat Inst.* (1951) 1. (2) *Fette und Seifen* 53 (1951) 332. (3) *The Vitamins*, Academic Press, New York, (in press). (4) *Archives Biochem. Biophys.* 37 (1952) 90; *ibid.* 41 (1952) 266. See also chapter on Autoxidation of Fats and Related Substances, section on Biological Significance of Lipids, p. 92.

However, BURR and collaborators,[20] using some of the same arachidonate as had been used by TURPEINEN,[23] were unable to confirm the fact that arachidonic acid exhibits a superior biopotency as compared with linoleic acid. Using an especially pure sample of methyl arachidonate and a new procedure for the bioassay of essential fatty acids, GREENBERG and co-workers[29] have recently reported that, when administered alone, methyl arachidonate possesses a biopotency 3·5 times that of linoleic acid. On the other hand, when the biological activity of arachidonate was calculated from the growth response observed when arachidonate and linoleate were fed together, the ratio of biopotencies of arachidonate to linoleate was 6·2 : 1. The weight of evidence would seem to indicate that arachidonic acid has the highest biopotency of the several essential acids.

4. *Procedures for determining the essential fatty acids*

a. Chemical methods for the determination of essential fatty acids

When a large proportion of the fatty acids present in a fat consist of one or more essential fatty acids, as occurs in the case of the common vegetable fats, quantitative analyses can be obtained by the use of fractional distillation under a high vacuum. Subsequent identification of the fractions can be made from saponification equivalents, iodine and thiocyanogen numbers, refractive indices, ultraviolet, infrared, or Raman spectral patterns or, in special cases, by the use of other physical or chemical constants.[42–44] Fractional crystallization of acetone solutions of the fatty acids at low temperatures, as developed in the laboratories of J. B. BROWN, have been useful in the separation of such naturally-occurring unsaturated acids as oleic, linoleic, linolenic, erucic, and ricinoleic acids.[45], [46]

The above procedures may be applied jointly to identify and to furnish quantitative data on the amount of unsaturated acids present, when they occur in comparatively large amounts. However, these methods are of little value for the estimation of the essential acids when the latter are present in the low concentration at which they occur in some vegetable fats, as well as in most animal fats. In the latter case, the procedure of BRICE *et al.*[47] has been most useful. This involves the isomerization of the unsaturated fatty acids which are non-conjugated to the corresponding conjugated acids, by heating with alkali. The concentration of each of the conjugated acids can be ascertained by spectrophotometric analysis.

However, the information obtained by chemical analysis may not always be valid. Dienoic, trienoic, and tetraenoic acids exist which do not possess biopotency, although they may contribute to the values obtained by spectrophotometric analysis. It would thus appear that the exact evaluation of the potency of an unknown oil, especially when the polyunsaturated acids are present in minimum amount, can be determined only by bioassay methods.

b. Bioassay procedure for the determination of essential fatty acids

A bioassay similar to that employed for vitamin A can be used to determine essential acids. That this procedure is valid became evident when GREENBERG *et al.*[29] demonstrated that the curve for the log dose plotted against gain-in-

weight, when doses of 5, 10, 20, and 50 mg of linoleate were administered to male rats having a fat deficiency, was a straight line after 3 weeks, continuing up to 12 weeks. The results of the control tests as recorded by GREENBERG and associates[28] are plotted in Fig. 1.

In tests on a group of hydrogenated fats (margarines and shortenings), butters, and cottonseed oil, in which the essential acids were determined both spectrophotometrically, by the procedure of BRICE *et al.*,[47] and by the bioassay procedure, there was a fairly good agreement in general. However, although the essential acid content of one shortening prepared by selective hydrogenation was very close when determined by the chemical and bioassay methods (2·1%

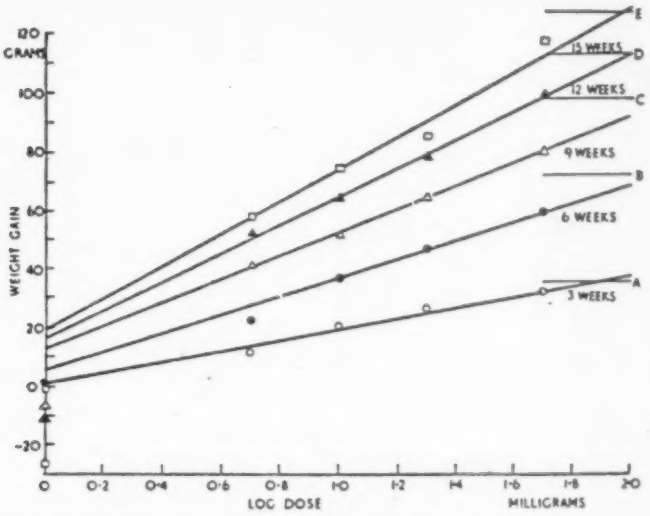

Fig. 1. The gain-in-weight of previously depleted male rats plotted against the log dose of methyl linoleate fed for 3, 6, 9, 12, and 15 weeks. Doses of methyl linoleate were 5, 10, 20, and 50 mg daily.[28]

vs. 2·46% respectively), a wide difference in values was obtained in another sample prepared by non-selective hydrogenation. In the latter case, only 6·8% of essential acids were found by the spectrophotometric procedure, while a figure of 13% was obtained by the bioassay procedure.[15]

5. *Essential fatty acid content of various fats and oils*

Linoleic acid is the main essential fatty acid found in vegetable oils, although in several instances (linseed oil and hempseed oil) large proportions of linolenic acid likewise occur. The essential fatty acids are usually present in highest concentrations in animal fats if large quantities of fats containing them have previously been fed to the animals. Small amounts of arachidonic acid have also been reported in many animal fats. In general, the amount of essential acids is lower in animal than in vegetable fats. Table 2 summarizes the maximum amounts of essential fatty acids which have been reported in the more common vegetable and animal fats.

Table 2. Relative maximum amounts of essential fatty acids in various vegetable and animal fats[48]

Vegetable fat	*Essential acids*		*Hydrogenated and animal fats*	*Essential acids*		
	Linoleic (%)	*Linolenic* (%)		*Linoleic* (%)	*Linolenic* (%)	*Arachidonic* (%)
Almond	19·9	—	*Hydrogenated fats*:[16]			
Avocado	10·3	—	Coconut oil	0	0	0
Brazil nut	22·8	—	Margarine oil I	5·0	0	0
Cocoa butter	21·1	—	Margarine oil II	5·8	0	0
Coconut	2·6	—	Margarine oil III	4·6	0	0
Corn	39·1	—	Margarine plus 1·5% cottonseed oil	5·1	0	0
Cottonseed	50·4	—	Shortening (selective)	2·2	0	0
Grapeseed	73·0	0·1	Shortening (non-selective)	7·1	0	0
Grapefruit seed	51·4	—				
Hackberry tree seed	76·7	—	*Animal fats*:			
Hempseed	68·8	24·3	Butter fat I[15]	3·2	0·9	0·2
Linseed	46·7	60·9	Butter fat II[15]	4·0	1·2	0·2
Olive	15·0	—	Egg yolk (hen)			
Palm	10.9	—	Hempseed oil diet	41·0	10·0	—
Peanut	27·4	—	Linseed oil diet	24·9	17·4	—
Perilla seed	33·6	—	Various diets	21·7	2·9	2·3
Pistachio nut	20·0	—	Phosphatides	8·2	—	—
Poppyseed	62·2	—	Goose fat (body)	19·3	—	—
Rapeseed	29·0	3·5	Hen fat (body)	21·3	—	0·6
Safflower seed	78·0	—	Human fat	11·0	—	1·0
Sesame seed	40·4	—	Ox depot fat	5·3	—	0·5
Soybean	58·8	8·1	Pig depot fat	15·6	—	2·1
Sunflower seed	68·0	—	Soybean oil diet	38·9	0·5	—
Tobacco seed	74·5	—	Peanut diet	19·7	—	—
Tomato seed	38·2	—	Cottonseed oil diet (12%)	26·8	—	—
Thorn apple	74·5	—	Cottonseed oil diet (8%)	18·2	—	—
Walnut	75·5	10·0	Cottonseed oil diet (4%)	13·3	—	—
Watermelon seed	65·8	—	Sheep depot fat	5·0	—	—
			Milk fat (cow)	5·8	—	0·4
			Milk fat (goat)	1·5	—	—
			Milk fat (human)	7·0	5·4	—

6. *Essential fatty acid requirement in animals other than the rat*

Although most of the experimental work on fat-deficiency has been carried out on the rat, there is every evidence that this dietary deficiency is a widespread one which may occur in a variety of different species. Thus, WHITE and co-workers[49] reported a syndrome in mice deprived of essential fatty acids which is similar to that produced in the rat. DECKER and his co-workers[50] have recently published extensive reports of essential fatty acid deficiency in mice. RUSSELL and his collaborators were unable to produce an essential fatty acid deficiency in chickens.[51] However, it is possible that the linoleic acid present in the cornstarch[1], [31] may have been sufficient to prevent severe deficiency symptoms. REISER[38] has recently shown the essential nature of polyunsaturated fatty acids by experiments in which he used more highly purified diets in which sucrose was substituted for starch as a carbohydrate energy source.

It has not been possible to produce the fat-deficiency symptoms in the hog, although HILDITCH, LEA, and PEDELTY[52] are of the opinion that the pig requires linoleic acid. However, the ELLIS group[53–56] reported that the linoleate content of lard dropped from a normal value of 7 to 15% to 1·2% when the diet of the hogs was practically fat-free. Moreover, when the diet contained vegetable oils high in essential fatty acids (such as soybean), the depot fat of the pig was found to contain as much as 39% of linoleic acid and 0·5% of linolenic acid.[55] A similar condition obtains in the egg yolk fat of the hen fed on hempseed oil.[57] GULLICKSON and collaborators[58] were unable to present any clear-cut evidence to prove that the essential fatty acids are required by growing calves; moreover, the results on the cow are similar,[59], [60] although it has been shown that a decrease in the iodine number of the blood lipids obtains when cows are subjected to fat-free diets.

The dog is readily susceptible to the fat-deficiency syndrome, and the course of the disease is quite similar to that in the rat.[61] Marked deviations occur in the proportion of the essential fatty acids in the plasma of this animal, as a result of variations in the intake of these components. The effect is especially evident when a fat-free regimen is given.

There are no definite indications that essential fatty acids are required by adult men, although several workers have indicated that these dietary components are required by babies. Thus, GRÖER[62] and HANSEN[63] have reported that infants kept on a low-fat diet developed eczema, which was readily cured by fat feeding. Although some other workers[64], [65] have failed to confirm these findings, as regards the curative effects of fat on infantile eczema, it is entirely possible that they may have been dealing with another type of eczema.

The evidence is quite definite that the eczematous condition can be correlated with the level of blood lipids. Thus, the scaly dermatitis which occurs in the rat on a fat-free regimen can be correlated with a lowering of the iodine number of the blood lipids.[66–69] Moreover, some workers[67], [70] reported that eczematous lesions in adult human patients are usually accompanied by a lowering in the level of unsaturated fatty acids, especially linoleate and arachidonate, in the serum. It is reported that, when the eczematous condition was cured by the

administration of lard, the improved clinical condition was associated with an increase in the linoleate content of the blood, but not necessarily with an augmentation of the arachidonate.[68] The data on the essential fatty acid content of blood serum of normal and of eczematous patients are summarized in Table 3.

Table 3. The linoleate and arachidonate in the blood serum of normal subjects and of eczematous patients[72]

Condition of patients	*Number of pooled samples*	*% of total acids*	
		Linoleate	*Arachidonate*
Young patients with eczema .	18	3·20	1·34
Adult patients with eczema .	8	4·20	1·60
Control subjects I . .	35	4·80	2·83
Control subjects II . .	12	5·20	2·90

Although it would appear probable that a correlation exists between the essential fatty acid level in the serum and the occurrence of certain types of eczema, in the baby and even in adult man, there is no other evidence which indicates the necessity of these substances in man. For example, BROWN and co-workers[71] carried out an experiment on a single normal male subject who maintained himself for six months on a low-fat diet; no harmful effects were noted, but a 50% reduction in blood linoleate and arachidonate was observed. This decrease was entirely out of proportion to the drop in total blood lipids.

It is apparent that the common animals can be divided into two categories. In one group, which includes mice, rats, and dogs, the deficiency of essential fatty acids in the diet is followed by cessation of growth, by kidney lesions, by effects on the epidermis and, in some cases, by death. In the second class, which includes pigs, calves and cows, and man, the only symptom which can be observed is the effect on the unsaturated acids in the blood which follows a prolonged fat-free dietary regimen. As would be expected from the results on rats, this effect is much more readily produced in the young animals than it is in the adults.

The reason for the differences between the two categories of animals is probably to be ascribed to variations in the level of requirement. In the rat the optimum level is extremely high, being in excess of 200 mg for a young adult male rat. If a similar requirement obtains for man, this would correspond to a value of 13·5 gm daily if comparative dosages are based upon surface area. One must conclude that the animals in the second group in all probability require essential fatty acids, but that the required level is considerably lower.

7. *Metabolic effects of essential fatty acids*

The main function of the essential fatty acids would appear to be related to the metabolism of the skin. Just how these acids act to carry on this function has not been determined.

One of the results of fatty acid deficiency which has been repeatedly recognized is the high level of metabolic activity which obtains. KUNKEL and WILLIAMS[73] have recently reported that essential fatty acid deficiency in the rat causes a marked increase in the cytochrome oxidase activity in the liver, as well as a slight augmentation in choline oxidase activity; no alteration occurs in the ex' :nt of succinic oxidase oxidation, but a marked decrease in endogenous ac ivity results. These authors are of the opinion that the increased cytochrome oxidase activity in the liver, and possibly also in the tissues, may largely account for the increased metabolic rate. It is believed that this may likewise represent a specific metabolic function of the essential fatty acids.

It is likewise possible that linoleic acid plays a role in the metabolism of choline. Thus, ENGEL[74] has suggested that this dienoic acid is essential to enable choline to exert its lipotropic effect. SMEDLEY-MACLEAN and NUNN[22] reported that fat was not laid down in body fat depots in the absence of arachidonic acid.

Another possible role of the essential fatty acids has been suggested by other work of SMEDLEY-MACLEAN and NUNN,[75] in which Walker tumour tissue was implanted in rats fed on a normal diet. Coincident with the development of the tumours, there was a marked fall in the ratio of highly unsaturated fatty acids to the fat-free dry weight in the subcutaneous tissues. This is interpreted to mean that, when new tissue is formed in the rat, arachidonic or some similar acid is used up in the promotion of this growth. The acid necessary for this process is drawn from the subcutaneous reservoir. The phenomenon of growth may well be associated, to a greater or lesser extent, with the metabolism of the essential fatty acids. The problem of the mechanism of the action of essential fatty acids in the animal body is thus as yet largely unsolved.

2. The Relationship of Fat Intake to Protein Metabolism

For a number of years, there has been a general agreement that carbohydrate rather than fat possesses a protein-sparing action. In the classical experiments of GRAHAM LUSK,[76] it was shown that, on a constant protein intake, the urinary nitrogen level was depressed when carbohydrate was ingested concomitantly, but not when fat was the additional foodstuff eaten. However, in more recent experiments, SWANSON and her co-workers[77] have been able to demonstrate that fat plays an important role in one phase of protein metabolism in which carbohydrate appears to be without effect. When rats were given protein-free diets over a sufficient period, until the urinary nitrogen had reached the so-called "wear and tear quota" or nitrogen minimum, the sparing action on protein continued when the caloric intake was reduced to 50% of that required, provided fat was included in the ration. Moreover, on the fat-containing diet, the nitrogen excretion was only slightly elevated when the caloric intake was reduced to 25% of that required. On the other hand, when the rats were fed a fat-free, protein-free diet, although the urinary nitrogen was not elevated when the diet was given at 75% of the requirement, a marked increase in protein metabolism was noted when only 50% of the caloric needs were satisfied. An

excessive loss of protein was demonstrated, as determined by urinary nitrogen values, when the diet furnished only 25% of the required calories. Similar results were also obtained when methionine was incorporated into the protein-free diet. These data are summarized in Table 4.

Table 4. Nitrogen excretion of rats receiving protein-free diet alone or with methionine and which was fat-free or contained 20% fat at 100, 75, 50, 25% of required caloric levels[77]

Per cent caloric requirement fed	*Basal N-excretion*		*Experimental N-excretion*		*Difference*	
	Fat-free diet	*20% fat diet*	*Fat-free diet*	*20% fat diet*	*Fat-free diet*	*20% fat diet*
Protein-free diet alone						
	mg	mg	mg	mg	mg	mg
100	280	295	185	210	− 95	− 85
75	272	310	211	263	− 61	− 47
50	283	265	594	289	+ 311	+ 24
25	249	290	818	544	+ 569	+ 254
Protein-free diet + methionine						
100	295	315	177	188	− 118	− 127
75	270	310	204	215	− 66	− 95
50	257	298	266	256	+ 9	− 42
25	281	329	577	523	+ 296	+ 194

Samuels, Gilmore, and Reinecke[78] have demonstrated the sparing action of fat on protein metabolism, by the use of an entirely new technique. Rats were fed on a diet containing 80% of the total calories as carbohydrate, protein, or fat for 28 days preceding a period of fasting. It was reported that the lowest nitrogen excretion obtained in the group which had previously received the fat diet. Moreover, the rats which had originally received the fat diet prior to the fast survived the longest. This phenomenon is, in all probability, to be ascribed to the greater sparing action of the fat on protein metabolism; the pre-mortal rise in protein metabolism is thus retarded, with a resultant prolongation of the survival period. The results of these tests are summarized in Table 5.

One possible explanation for the striking results given in Table 5 is that the previous dietary history conditions the enzyme systems which are active during a subsequent fast period.[79] In the case of the rats which have received exogenous fat as the sole source of calories during the 28-day period prior to the fast, the enzyme systems are completely adjusted to the processes involved in the efficient utilization of fat. When the supply of fat is endogenous rather than exogenous, no alterations in oxidation pattern are involved, and the same metabolic processes proceed without interruption. In the case of the rats which have previously received either the protein or the carbohydrate regimen, a new

Table 5. The average nitrogen excretion of rats on special diets and during a subsequent period of fasting with voluntary activity, and of rats undergoing violent exercise[78]

Category	Period during fast	Nitrogen excretion in gram/day/rat*		
		Protein diet	Carbohydrate diet	Fat diet
	(days)			
Total nitrogen excretion	Before (on diet)	1·369 ± 0·38	0·3375 ± 0·0053	0·3165 ± 0·0080
	0–4	0·2547 ± 0·0314	0·1770 ± 0·0068	0·1104 ± 0·0057
	6–11	0·1427 ± 0·0392	0·1318 ± 0·0111	0·1004 ± 0·0094
	13–death	—	0·2286 ± 0·0131	0·1407 ± 0·0272
Nitrogen excretion during work (mg/100 revolutions)	—	0·0521 ± 0·0095	0·0465 ± 0·0058	0·0342 ± 0·0044

* Including Standard Error of the Mean.

pattern of oxidation must be set up as soon as the glycogen stores and stores of deposit protein are exhausted. Finally, the shorter survival of the rats which had previously received the protein diet might be explained by the fact that the animals were unable to adjust themselves to an economical use of protein during fasting because they had become accustomed to a high protein intake with a large wastage of this foodstuff in the pre-inanition period.

Another approach to the question as to the protein-sparing action of fat is the study of efficiency of growth in young animals on low-fat and on high-fat diets. FRENCH, BLACK, and SWIFT[80] have reported more efficient and more rapid gains-in-weight in rats receiving a low-protein diet when 30% of fat was present than when only 2% of this foodstuff was involved. GEIGER[81] has recently shown that the sparing effect of cystine on protein metabolism occurs only when fat is present in the diet. This confirms an earlier report of SALMON,[82]

ROGERS, FERGUSON, FRIEDGOOD, and VARS,[83] experimenting with protein-depleted rats which were then subjected to 70% partial hepatectomy, found that the nitrogen balance was more favourable and that greater increments of liver protein accrued in rats kept on a diet composed of 30% of fat than on one containing 3% of this foodstuff. Such results were likewise obtained when the caloric intake of the animals on the 30% fat diet was restricted to the *ad libitum* consumption of the 3% fat regimen.

A possible explanation for the behaviour of fat on protein metabolism may be deduced from the experiments of PEARSON and PANZER.[84] These workers reported that a significantly greater loss of phenylalanine, valine, lysine, and methionine obtained in the urine and faeces of growing rats on a fat-free diet than on one which contained 8% of corn oil. Moreover, similar but less striking differences in favour of the fat diet were registered in experiments with adult rats.

The report of SCHWIMMER and MCGAVACK[85] indicates that the protein-sparing action of fats may likewise have application to man. It was found that

the only condition under which a low nitrogen excretion could be maintained in human subjects on protein-free diets, fed at considerably below the levels required for caloric equilibrium, was when the rations contained considerable proportions of fat. Although one must continue to accept the fact that carbohydrate serves to spare protein on mixed diets, it seems necessary to include an additional corollary that fat and fat alone may likewise exert a protein-sparing action when animals are subjected to low-protein or protein-free diets.

3. Growth as Related to Fat Intake

1. *Experiments in which ad libitum diets were employed*

There is considerable evidence in the literature to prove that the growth-promoting properties of diets are progressively improved as the proportion of

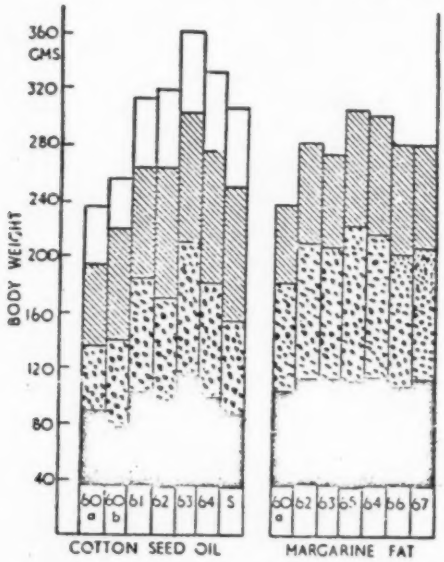

Fig. 2. The average body weight of weanling male rats at the start (to top of lower blank space), after 3 weeks (to top of solid black), after 6 weeks (to top of stippled area), after 12 weeks (to top of cross-line area), and after 18 weeks (to top of upper blank area). The figures in the lower blank area are the diet numbers (described in text).[88]

fat is increased. HOAGLAND and SNIDER[86] were the first to prove that, on diets containing 5, 30, or 55% of four types of lard, rats grew best on the regimens containing 30% and 55% of fat. In later tests carried out with increased proportions of vitamins, these workers[87] confirmed their earlier results; when steam-rendered lard was fed at 5, 15, 30, or 54% levels in the diet, optimum growth obtained in the animals receiving the 30% fat diet, and minimum growth was noted in the group receiving the 5% fat diet. DEUEL and co-workers[88] have also noted that weanling rats fed on diets containing several levels of cottonseed oil or margarine fat showed the best growth when the fat comprised 20 to 40% of the total weight of the diet. The authors suggest that the optimum

level of fat as estimated from these tests would be approximately 30% by weight, or 50% of the calories.[89] Fig. 2 illustrates the mean body weights, at several periods, of weanling rats fed over an 18-week interval on 0% fat without (diet 60a) or with (diet 60b) essential fatty acids, or with diets containing fat at

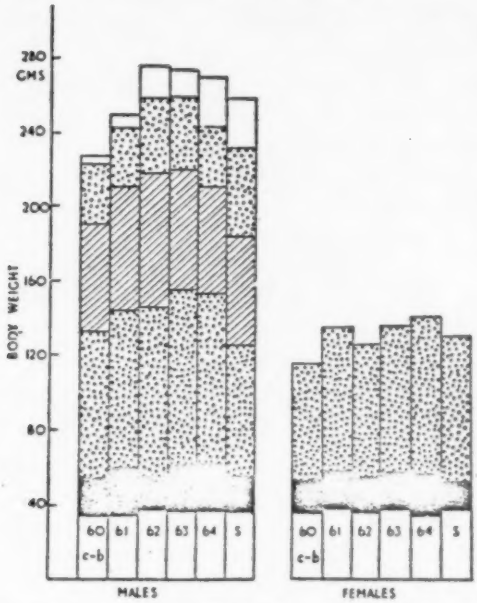

Fig. 3. The average body weight of weanling male and female rats at the start (to top of lower blank space), after 12 weeks on restricted calories (to top of solid black area), after 3 weeks on diets *ad libitum* (to top of stippled area), after 6 weeks on diets *ad libitum* (to top of cross-lined area), after 9 weeks on diets *ad libitum* (to top of upper stippled area), and after 12 weeks on diets *ad libitum* (to top of upper blank area).[90] The fat was cottonseed oil.

the following levels: 5% (diet 61); 10% (diet 62); 20% (diet 63); 30% (diet 65); 40% (diet 64); 50% (diet 66); and with a mixture of margarine fat (7·2%) and cottonseed oil (2·8%) (diet 67) and with the stock diet (S).

2. *Experiments in which paired-feeding tests were employed*

In the experiments of FORBES and co-workers[91] carried out with pair-fed rats on isocaloric diets containing 2, 5, 10, or 50% of fat (mostly lard), the gains-in-weight, the digestibility of the fat, and the retention of nitrogen displayed proportionally greater efficiency as the amount of fat in the diet was increased. In another series of tests on growing rats, carried out with 2, 10, and 30% fat but with increasing proportions of vitamins as compared with that in the earlier series, FORBES and collaborators[92] proved that the greater efficiency of the diets with increasing fat content was associated with significant gains of fat and energy and with a decrease in the total heat production. In experiments with mature rats, again employing diets containing 2, 10, and 30% of fat, FORBES *et al.*[93] noted that the fat content of the diet had little effect on the utilization of protein, but that the energy loss associated with the utilization of isocaloric

quantities of the diets decreased with the increasing fat content of the ration. In an extension of this work, FRENCH and co-workers[80] demonstrated that the high-fat diet (30%) produced better results, from the standpoint of energy utilization, than did the low-fat diet (2%), when the protein level was reduced from 22% to 7%. There was an increased weight gain; furthermore, greater increases in fat content and energy, and a decrease in heat production, were associated with the high-fat regimen.

In contrast to the uniformly superior results obtained with high-fat as compared with low-fat diets, which have been recorded by a number of investigators, HOAGLAND, SNIDER, and SWIFT[94] have recently been unable to find statistically significant differences in the rate and efficiency of growth of young male rats fed diets containing 4, 9, or 14% of the diet (5·0, 10·98, or 18·27% of the calories respectively).

3. *Experiments on fat feeding following periods of restricted food intake*

The improvement in nutrition and growth which results from a generous inclusion of fat in the diet has likewise been demonstrated by SCHEER *et al.*,[90] who varied the experimental conditions. When weanling rats were stunted by drastic restriction of the food intake to such an extent that practically no increase in body weight occurred over a period of 12 weeks, and the rats were then allowed to eat the various diets *ad libitum*, markedly superior growth was exhibited by the rats receiving 10, 20, and 40% of fat in the diet, as compared with that recorded for the animals receiving 0% or even 5% of cottonseed oil. This was particularly evident during the last period investigated (9 to 12 weeks after the start of *ad libitum* feeding).

Essentially similar results were reported by SCHEER *et al.*[95] in later studies on young adult rats. When these rats were subjected to severe caloric restrictions, the loss-in-weight of the rats receiving the high-fat diets was less than in the case of those receiving isocaloric low-fat diets. During the period of caloric restriction, a lower mortality rate was likewise observed in the group of rats receiving the high-fat diet than in those on the low-fat regimen. Moreover, the rats on high-fat rations made a better recovery during a subsequent period when *ad libitum* feeding was reinstituted than did those on the low-fat diets. Finally, PEARSON and PANZER[84] have shown that rats fed a diet containing 8% of corn oil or lard *ad libitum* gained 29% more in body weight than did rats maintained on a similar diet minus the fat but with the addition of ethyl linoleate.

4. *"Associative dynamic action" as an explanation of the greater efficiency in utilization of fat diets*

The best explanation for the greater efficiency in growth which has been demonstrated for high-fat diets, as compared with isocaloric low-fat regimens, is the peculiar effect exerted by fats on the specific dynamic action of other foodstuffs. This behaviour of fats, which was first discovered by FORBES and SWIFT,[96] has been termed by them the "associative dynamic effect."

It has long been known that, following the ingestion of foodstuffs, an increased

respiratory metabolism obtains over and above the basal metabolic level, during the period of their absorption, transport, and interconversion in the body. This is known as the "specific dynamic action." Proteins exert the greatest effect in this respect, followed, in turn, by carbohydrate and fat. Under certain conditions, such as external cold, the heat arising from the specific dynamic action of the foodstuffs may be utilized for warming the body, in lieu of a stimulation of the metabolism by shivering or by chemical regulation, and may thus produce the extra heat required to accomplish this purpose.[97] Under these circumstances, the specific dynamic effect would not represent waste energy, but would rather tend to result in greater efficiency in the utilization of foodstuffs. However, under most conditions, the specific dynamic effect represents a direct loss of energy to the body. When the specific dynamic effect is not needed for warming the body, due to an elevated environmental temperature, this excess heat is lost. Moreover, the energy produced by the specific dynamic effect of protein cannot be used to derive energy for mechanical work.[98, 99] However, ANDERSON and LUSK[99] did demonstrate that, when carbohydrate was consumed during work, no specific dynamic effect was exerted. There is no conclusive evidence as to the effects which would be noted, under these conditions, with ingested fat. Thus, it should become apparent that any situation which tends to reduce the specific dynamic effect of foodstuffs will bring about an increased efficiency in utilization of the foodstuffs.

It has been generally accepted that, under conditions favourable for demonstrating the specific dynamic effect, the activity due to each of the foodstuffs is additive. Some years ago, MURLIN and LUSK[100] demonstrated, in tests on dogs, that the extra heat produced when glycine (20 gm), glucose (70 gm), and fat (75 gm) were fed together was practically identical with the sum of the specific dynamic effects obtained when each of the foodstuffs was fed separately. Although the data of MURLIN and LUSK appear quite conclusive, it is possible that the deductions may have been erroneous, since the calculations were based only upon a 2-hour period (at which time the specific dynamic action was believed to be at a maximum) rather than upon the entire period during which the effect was exerted. Moreover, one might likewise question whether or not the period of maximum extra heat production might have been modified when the three foodstuffs were given together, since the presence of fat might be expected to alter the rate at which protein and carbohydrate could be absorbed.

In their studies on rats, FORBES and SWIFT[96] have overcome these criticisms by determining the heat production over the entire period during which the dynamic effects were being exerted. The new procedure, according to these workers, is more representative of common nutritive practice, as compared with the measurement of the dynamic effects of single test meals. Moreover, the results are more significant, and are probably more accurate, inasmuch as they are determined as differences between the amounts of heat produced at established levels of metabolism, without concern as to the beginning, maximum or termination of the specific dynamic effects of a single meal. Finally, no confusion arises in regard to the dynamic effects of the catabolized body nutrients.

The specific dynamic effects per 1000 calories were found to be as follows when the foodstuffs were fed separately to rats: beef protein, 323 calories; glucose, 202 calories; and lard, 160 calories. When glucose and protein were fed together, the response obtained agreed quite closely with the additive effects of the two foodstuffs calculated from the previous figures for the quantities of each foodstuff fed. On the other hand, whenever lard was fed, the calculated values were always considerably greater than those actually found. Thus, when beef protein and lard were fed simultaneously, only 113 extra calories were produced per 1000 calories fed, compared with a computed value of 244 calories. This sparing effect was likewise noted to a somewhat lesser degree when fat was fed with glucose alone or with glucose and protein. It would appear that fat was the only one of the three foodstuffs to exhibit this so-called "associative dynamic effect." A graphic presentation of the data of FORBES and SWIFT[96] is given in Fig. 4, while Table 6 supplies a breakdown in the partition of the average daily intake of energy by the rat.

Table 6. Partition of average daily intake of energy per rat during 70 days[101]

Category	*Diets containing fat at levels of*			
	2%	5%	10%	30%
Energy intake, *calories*:				
Gross	2854	2854	2854	2854
Protein	799	799	799	799
Carbohydrate	1912	1713	1456	657
Fat	143	342	599	1398
Metabolizable calories	2601	2605	2610	2615
Energy output, *calories*:				
In faeces	128	126	121	123
In urine	125	123	123	116
As heat	2195	2165	2154	2155
Energy retained, *calories*	406	440	456	460

The decreased expenditure of total calories which occurs when fat replaces carbohydrate in isocaloric diets is sufficient to account for the greater efficiency with which high-fat diets are utilized for growth, as compared with low-fat diets. Although the authors do not pose an explanation as to the cause of the effect of fat on specific dynamic action, it is possible that the prolongation in the absorption period of protein and carbohydrate would lessen the maximum concentration of these components, and thus reduce their specific dynamic action. FORBES *et al.*[96] suggest that it is thus not necessary to diminish the protein content of the diet during hot weather in order to ensure a low heat increment; *rather one need only substitute fat for some of the carbohydrate. However,*

there is no proof that fat exerts an "associative dynamic action" in man as it does in the rat.

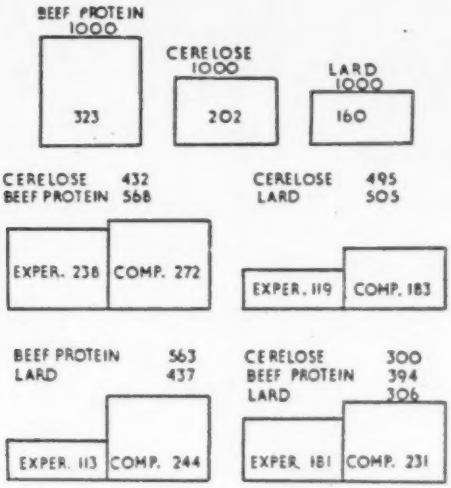

Fig. 4. Dynamic effects per 1000 calories of gross energy of nutrients as affected by nutrient combination.[98]

5. *Sexual maturity as related to dietary fat*

Although sexual maturity is not as obvious an indication of growth as gain-in-weight, nevertheless it is a factor which must necessarily be dependent upon this physiological change. The completion of sexual maturity can be determined with reasonable precision in the female rat from the opening of the vagina. The time at which sexual maturity occurs has not ordinarily been considered as one of the indices of nutrition, but there is no reason why it should not afford a satisfactory indication of this state.

In the experiments of DEUEL and co-workers,[88] it was shown that, whereas normal female rats did not become sexually mature, on a fat-free diet, until they were approximately 81 days of age, maturation took place as early as 70 days

Table 7. The effect of fat level in the diet on the time when sexual maturity develops in the female rat

Previous diet	*Average time in days to maturity after start of* ad libitum *feeding in diets containing:*				
	0% *fat*	5% *fat*	10% *fat*	20% *fat*	40% *fat*
Normal rats, 3 weeks old at start, on diets *ad libitum* from weaning[88]	60·2	49·0	45·5	44·8	44·1
Restricted rats, 15 weeks old at start, after 12 weeks on restricted calories, and then given diets *ad libitum*[90]	14·7	11·9	10·5	7·7	9·8

after birth in the group receiving 5% of fat in the diet, and at 65 days in the animals receiving the 40% fat regimen. In rats which had received calories restricted sufficiently to prevent appreciable growth over a 12-week period following weaning, sexual maturity did not obtain in any case, irrespective of the amount of fat in the diet. However, when the rats had been allowed to consume the diet *ad libitum*, sexual maturity occurred most promptly in the rats on the 20% fat ration, followed by those on the 40% fat regimen. The data are summarized in Table 7.

4. Pregnancy and Lactation as Indices of the Nutritional Value of Fat

The opinion is practically unanimous among nutrition workers that pregnancy offers a better index of the nutritional value of a diet than does growth alone. It is also generally agreed that a still more precise evaluation of the nutritive value of a foodstuff may be obtained from an examination of the response of lactation.

A number of different workers have reported that reproductive failure invariably ensues in rats raised to maturity on fat-free diets.[102–104] After a prolonged gestation period and excessive vaginal bleeding, the young are generally born dead, or they die shortly after birth. However, QUACKENBUSH and his co-workers[104] found that, if the female rats on the fat-deficient diet were given as small an amount as 100 mg of ethyl linoleate 3 weeks before they were bred, a normal gestation period was noted, and up to 83% of the young were weaned. KUMMEROW and associates[105] reported that, when the fat-free diet was replaced by 5% hydrogenated fat, the animals gave birth to living young which did not live more than 72 hr; however, when the diet contained 5% of corn oil, 85% of the young were weaned. The mothers and newly-born young were deficient in arachidonic acid, but not in fat *per se*. The main differences occurred in the phospholipid fraction of the young. After corn oil, the phospholipids in the young contained 5 to 10 times more arachidonic acid than did those from rats on a fat-free diet. The phospholipids from rats which had received hydrogenated fat contained twice as much of the acid as did those from the rats on a fat-free regimen.

Although it is obvious that marked differences obtain in pregnancy and lactation on fat-free diets, as contrasted with the results on fat diets, it is somewhat more difficult to demonstrate variations in these physiological functions when different levels of dietary fat are fed. Thus, no differences in reproductive and lactation performance could be noted in normal rats receiving 5 to 40% of fat. The results in all cases were found to be superior to those obtained on a fat-free diet.[88] However, when rats were first subjected to a 12-week period of undernutrition followed by an *ad libitum* feeding period, SCHEER *et al.*[90] reported that the best performance (both in the number in the litter and in the weaning weights) was obtained in the group receiving 40% of cottonseed oil in the diet. The data based upon these experiments are recorded in Table 8.

Although the weaning weights of the individual rats do not exhibit very great differences, the total litter weight of the rats receiving the 40% fat diet is

Table 8. Reproductive performance of female rats recovering from a period of undernutrition[90]

Diet		Average number rats per litter	Average litter weight		Average rat weight	
Number	Fat content		3 days	21 days	3 days	21 days
	%		gm	gm	gm	gm
60b*	0	5·3	47·6	152	8·9	26·1
61	5	6·8	47·7	219	7·1	32·4
62	10	7·7	64·3	167	8·4	27·8
63	20	8·5	60·8	230	7·2	31·8
64	40	8·9	72·3	283	8·1	30·6

* Containing linoleic acid.

approximately twice that of the rats receiving the fat-free diet supplemented with linoleate. Confirmation of the improved effect of high-fat diets on pregnancy was obtained by SCHEER *et al.*[95] in another series of tests in which adult rats were first subjected to caloric restriction to such a degree that the animals lost approximately one-half of their body weight over a 12-week period. After this interval, the animals were continued on the diets containing the several fat levels, but the food was given *ad libitum*. Breeding tests were conducted immediately following recovery. The following percentage of fertility was noted in the groups on the several diets: 0% fat, 20%; 5% fat, 0%; 10% fat, 20%; 20% fat, 80%; and 40% fat, 80%. However, in no case were the mothers able to raise their progeny to the age of 21 days.

The evidence afforded by a number of types of experiments would seem to indicate the great importance of fat in rendering normal pregnancy and lactation possible. To what extent this favourable effect is to be traced to the essential fatty acid content of fat is not known. Although a 5% cottonseed oil diet would provide more than the required linoleate for the female rats, the best performances in pregnancy and lactation have been recorded when 20% or 40% cottonseed oil diets were given. Therefore, it would appear that some factor in fats, in addition to the essential fatty acid content, is responsible for their beneficial effect under these conditions.

5. Work Capacity as an Index of the Nutritional Value of Fat

Although carbohydrate has long been regarded as the main source of energy for muscular exertion, a number of observations indicate that fat may play an even more important role in this physiological function. KROGH and LINDHARD[106] reported, many years ago, that work could be accomplished by man approximately 10% more efficiently when carbohydrate served as a source of calories than when fats were exclusively metabolized. On the other hand, these results entirely contradicted the exhaustive studies of ANDERSON and LUSK,[99] which were carried out with dogs. The latter investigators reported that, from a caloric standpoint, dogs ran with equal efficiency in the respiration calorimeter,

irrespective of whether they had a Respiratory Quotient of 1·0 and so were using carbohydrate almost exclusively, or whether they had a non-protein R.Q. of approximately 0·71, which would indicate that fat was being used almost exclusively. The latter condition was attained after a 13-day fast. In view of the conflicting data of these two groups of investigators, the question of the source of fuel for muscle work can still not be considered as a settled problem. Either species difference accounts for the variation (and this would seem improbable), or the conditions in one of the experiments were not adequate for testing the hypothesis.

More recently, the problem has been reinvestigated, using total working capacity as the index for comparison. When rats receiving diets containing several levels of cottonseed oil or margarine fat were subjected to exhaustive swimming tests, it was found that those on the higher fat levels had a greater capacity for work. The procedure for testing physical capacity, as developed by SCHEER, DORST, CODIE, and SOULE,[107] was employed. This involved the determination of the duration of the rats' capacity for swimming when increasing loads were given to them at successive 3-minute intervals. All animals had been adjusted to the same specific gravity before the start of the test. The results of these tests are summarized in Table 9.

Table 9. The effect of fat level of the diet on the working capacity of rats, as determined by a swimming test[88]

Sex	*Period on diet*	*Duration of swim in seconds on diets containing the following per cent of fat (by weight):**								
		60a 0%	60b 0%	61 5%	62 10%	*Stock* 14%	63 20%	65 30%	64 40%	66 50%
Cottonseed oil diet										
	weeks									
Female . .	6	512 (5)	580 (5)	802 (6)	777 (5)	1025 (4)	*1062* (5)	—	922 (3)	—
Male . .	6	805 (5)	692 (5)	1019 (5)	921 (5)	955 (4)	1223 (5)	—	1142 (5)	—
Male . .	12	645 (20)	637 (19)	769 (19)	*850* (17)	*1084* (19)	846 (19)	—	*1068* (15)	—
Margarine fat diet										
Male . .	12	485 (23)	—	—	*806* (10)	—	*1174* (8)	*1065* (25)	876 (4)	*1122* (10)

* The figures in parentheses represent the number of experiments included in the average. Values in italics were shown to be statistically greater than those on diets 60a and 60b.

The work capacity was shown to be statistically greater in most cases in which there were sufficient tests to support this mathematical evaluation on the fat-containing diets, as contrasted with the performance on the fat-free regimen.

Furthermore, longer periods of work were noted in some cases in which higher fat diets were used than when the fat was present in lower amounts. Thus, it was found that the rats receiving the 20% level of margarine fat (diet 63) did bett- than those on the 10% level (diet 62) which, in turn, had a statistically greater physical capacity than did the animals on the fat-free diet (diet 60a).[88] In later experiments by SCHEER and collaborators,[107] it was shown that physical capacity decreased markedly when the caloric intake was severely restricted. Under such conditions, the level attained was independent of the fat content of the diet.

SAMUELS, GILMORE, and REINECKE[78] have compared work capacity and survival of rats during fasting, following a 28-day period throughout which the rats had received diets containing 80% of the calories from fat, carbohydrate, or protein. Not only was spontaneous activity in general greater in the case of the rats which had previously received the fat diet, but also the total forced activity in this group greatly exceeded that in the other groups. The latter phenomenon is partly to be ascribed to the longer period of survival by the fat-fed group. These data are summarized in Table 10.

Table 10. Effect of previous diet on spontaneous and forced activity and on the period of survival of rats during fasting[78]

Category	*Previous diet**		
	Fat	*Carbohydrate*	*Protein*
Spontaneous activity, cage revolutions:			
2–4 days	6730 ± 915 (12)	4040 ± 411 (16)	3600 ± 262 (7)
9–11 days	8800 ± 1020 (13)	8660 ± 1017 (14)	7220 ± 500 (4)
16–18 days	7960 ± 738 (7)	8650 ± 0000 (3)	— (0)
Forced activity, cage revolutions	19700 ± 1780 (12)	13800 ± 1630 (14)	6465 ± 1650 (9)
Survival time, days	18·3 ± 0·44 (12)	15·5 ± 0·46 (13)	10·2 ± 1·68 (9)

* Including Standard Error of the Mean. The figures in parentheses designate the number of the rats in each group.

6. Survival Time as an Index of the Nutritional Value of Fat

The length of life and the duration of survival during fasting are two nutritional indices which are of importance in the evaluation of the nutritive value of a foodstuff. Although there are no data available to answer the question as to whether or not the high-fat diets have a superior effect on longevity as compared with other foodstuffs, the recent experiments of SAMUELS, GILMORE, and REINECKE[78] have indicated that fat is the best protective foodstuff in preparation for a subsequent fast period. When rats were fed on diets containing 80% of fat, carbohydrate, or protein for 28 days prior to fasting, the survival during the period of inanition was statistically greater when the pre-fast diet was fat

than when it was carbohydrate, while the latter foodstuff was again distinctly superior to protein as judged from the survival time. These data are summarized in Table 10. The longer survival time following pre-feeding with fat, as compared with that after the administration of protein or carbohydrate, is probably to be traced to the fact that a metabolic pattern is developed during the pre-inanition period, when fat is given, which is especially favourable for a prolonged survival. SAMUELS *et al.*[78] showed that a marked sparing action affecting the protein metabolism obtained during the fasting period when fat had been the previous food, as compared with that when the pre-inanition regimen had consisted of carbohydrate.[108] ROBERTS and SAMUELS[109], [110] reported that rats which had become accustomed to a high-fat diet had a markedly lower susceptibility to insulin injection than did animals which were receiving a high-carbohydrate ration. This resistance to insulin on the part of the fat-fed rats was ascribed to a prolonged retention of liver glycogen in these animals; it was later suggested[111] that rats on a diet in which 85% of the calories are derived from fat develop a sparing action for liver glycogen. In support of this theory, it was found that hepatectomized rats which had previously received a fat regimen were free from hypoglycemic symptoms for a longer period after the operation than were rats which had received a high-carbohydrate diet prior to the removal of the liver.

In experiments on isolated tissues, GILMORE and SAMUELS[79] noted that a difference in glucose utilization obtained in tissues removed from animals previously on the fat diet and from rats in which carbohydrate had been the pre-fasting regimen. These experiments support the earlier data of LUNDBAEK and STEVENSON,[112] who reported that the diaphragm of a carbohydrate-fed rat utilized glucose *in vitro* at approximately twice the rate which occurred in the muscle of the fat-fed rat, when both tissues were immersed in a glucose medium.

TEMPLETON and ERSHOFF[113] investigated the relative influence of the several foodstuffs on survival, making use of another procedure. When the stress employed was thyroxine, it was found that rats fed fat (margarine fat) or carbohydrate (sucrose) lived appreciably longer than those which received protein (casein). No significant differences were noted in control tests when saline was injected instead of the thyroxine solution. Moreover, when another stress was used, namely a low temperature, and the rats on the several diets were exposed to an environment of 2°C, the fat-fed and carbohydrate-fed rats had greater resistance than did the protein-fed animals. On the other hand, no difference related to diet was observed when the temperature to which the rats were exposed was 23°C.

7. The Effect of Dietary Fat on Vitamin Requirements

1. *Thiamin and fat*

It has long been recognized that the metabolism of thiamin and that of fat are interrelated. As early as 1928, EVANS and LEPKOVSKY[2], [114] discovered that, on diets adequate except for their content of vitamin B_1, the onset of polyneuritic

symptoms was retarded on high-fat diets; on the other hand, when high-carbohydrate diets were employed, polyneuritis developed at an accelerated rate. Proof that the behaviour of fat is a generalized effect was afforded by the fact that many vegetable and animal fats were found to be equally efficacious in retarding the onset of polyneuritis. Thus lard, coconut oil, synthetic coconut oil, hydrogenated coconut oil, partially hydrogenated cottonseed oil (Crisco), cottonseed oil, and perilla oil were all effective.[115], [116] The only fat which did not possess a thiamin-sparing action was hydrogenated perilla oil; this product, which melted at 67·5°C, was believed to be inactive, because of its low digestibility.[116]

In addition to digestibility, several other factors are of importance as related to the sparing action on thiamin. In the first place, unsaturation *per se* does not seem to play a role, except when complete saturation of a fat is associated with its defective absorption, as has already been mentioned. Thus, EVANS and LEPKOVSKY[116] found that fats having an iodine number of only 8 spared thiamin as effectively as did a highly unsaturated oil with an iodine value of 187. Such saturated triglycerides as tricaprylin and trimyristin were found to be very effective in sparing vitamin B_1, although tristearin was inactive.[117] The latter fat is now known to be practically unabsorbable by the rat.[118], [119] According to EVANS and LEPKOVSKY,[116] neither the precise melting point nor the degree of saturation of the several other natural fatty acids appears to influence this activity. On the other hand, SALMON and GOODMAN[120] demonstrated that the variations in the thiamin-sparing action could be demonstrated in synthetic esters of the fatty acids; the maximum vitamin-sparing action was found with the C_8-fatty acid ester.

In addition to the fact that the symptoms of polyneuritis are delayed in rats by the presence of fat in vitamin B_1-free diets, it has also been shown that other symptoms associated with thiamin deficiency are retarded by this dietary procedure. Thus, MACDONALD and MCHENRY[121] were able to demonstrate, in 1940, that the onset of bradycardia, which is characteristic of thiamin deficiency in the rat, was also postponed when fat was included in the diet. It was likewise shown that another symptom of thiamin deficiency which is typical of the polyneuritic syndrome, namely the augmented excretion of bisulphite-binding substances, is also modified by the presence of fat in the diet.[120–123] This latter effect does not appear to be caused by an interaction between the fat and thiamin in the intestine, since it is also observed when the thiamin is administered parenterally.[124] Later experiments of BANERJI and YUDKIN[123] demonstrated that kidney slices from rats on different diets invariably showed a defective oxidation of pyruvate, which could be corrected by the addition of thiamin to the respiring tissue. This condition obtained irrespective of the diet of the rat from which the tissue had been removed. It is therefore evident that the behaviour of fat in inhibiting the production of the pyruvate (bisulphite-binding) compounds is to be attributed to a decrease in the metabolism of the substances from which the compounds are derived rather than to an alteration in the pathway followed by their oxidation. According to MELNICK

and FIELD,[125] fats which show the sparing action do not contain any thiamin.

Apparently the presence of fat in the gastrointestinal tract can influence the bacterial synthesis of thiamin. Thus, WHIPPLE and CHURCH[126] were able to show that, on a thiamin-deficient diet, the faeces of rats contained some thiamin if lard was incorporated into the diet. However, when the lard was omitted from the ration, the thiamin activity of the stools was completely lost. In human subjects, carbohydrate ingestion has been shown to cause a decrease in the output of thiamin in the urine, which would seem to indicate that a greater utilization of thiamin occurs under these conditions. On the other hand, in spite of the fact that a high-fat diet reduces the thiamin requirement in experimental animals,[2], [127], [128] it has been impossible to demonstrate that such increases in fat consumption augment the urinary excretion values of this vitamin.[129], [130]

In later work, WHIPPLE and CHURCH[131] have adduced evidence to indicate that thiamin has a function in the synthesis of fatty acids from carbohydrate. The demonstration that this transformation is continually taking place may be attributed to SCHOENHEIMER and RITTENBERG,[132] who carried out experiments in mice, using tracer substances. These authors have shown that there is a rapid turnover not only of the phosphorus-containing lipids of the kidney and liver but also of the fat deposits; this necessitates a continuous conversion of carbohydrates into fatty acids under normal dietary conditions. If, when fat is fed, the extent of the carbohydrate → fat conversion is reduced, one might naturally expect thiamin to be spared. SCHOENHEIMER and RITTENBERG[132] look on the carbohydrate → fat change as a normal physiological method whereby the nutrients are stored until needed for oxidation. Inasmuch as food is taken only at intervals in the case of most animals, it must be deposited for a short period before it is further metabolized. Since there are insufficient facilities for storing the excess carbohydrate as glycogen, most of the excess carbohydrate is immediately changed to fatty acids. These are temporarily laid down in the fat depots, to be utilized for absorption during the post-absorptive periods.

2. *Riboflavin and fat*

The interrelation between fat and riboflavin (vitamin B_2) is not as clear-cut as in the case of thiamin. EVANS *et al.*[3] suggested in 1934 that fat has no sparing action on the riboflavin requirements in the rat comparable to that observed with thiamin. On the other hand, MANNERING and his co-workers[133], [134] have postulated that actually more riboflavin is required by rats when they are receiving a high-fat diet than when they are on a low-fat ration. A possible explanation for the augmentation of the riboflavin requirement in the presence of high-fat diets, which has been suggested by ELVEHJEM,[135] is that the intestinal synthesis of riboflavin is inhibited when fat is substituted for dextrin in the diet. On the other hand, CZACZKES and GUGGENHEIM[136] reported that rats maintained on a low-protein diet excreted so much riboflavin that they failed to maintain a functional level in their tissues, and ultimately died of ariboflavinosis.

When the protein or fat content of such diets was increased, the excretion of riboflavin was reduced, while a decreased fat content increased the excretion. These authors attribute such variations to differences in the extent of synthesis of this vitamin in the intestinal flora. It was shown that rats on high-protein and high-fat diets need at least twice as much riboflavin for the maintenance of the riboflavin level in the organs and in the urine as do rats kept on a "normal" diet. POTTER and associates[137] have been unable to show a similar increase in riboflavin requirements on high-fat diets when puppies were the experimental animals.

Fat has been shown to have a stimulatory effect on the growth of bacteria, as demonstrated by microbiological methods for the determination of riboflavin.[138], [139]

3. *Pantothenic acid and fat*

Deficiencies in pantothenic acid, pyridoxine, and essential fatty acids result in the production of forms of dermatitis which are similar in appearance. According to SALMON,[140] the actions of all of these essential components are related, and all three are required to cure the dermatitis caused by pantothenic acid deficiency.

STOTZ[141] has suggested another role for pantothenic acid. According to this investigator, the vitamin in the form of a co-enzyme A, called co-acetylase, may be involved in fatty acid synthesis. The presence of this enzyme, now known as "acetylase," was first proved by LIPMANN and collaborators.[142] These workers are of the opinion that acetylase is important both in pyruvate and in acetate metabolism.[143], [144] Moreover, SOODAK and LIPMANN[145] have deduced proof that the co-enzyme A is required for the formation of acetoacetate. All these considerations lead one to the conclusion that pantothenic acid is of importance in the biosynthesis of fat in the animal body. The amount of this vitamin required is obviously a function of the amount and type of fat in the diet.

In the microbiological assay for pantothenic acid, fat exhibits the same stimulating effect that it does in the case of the assay for riboflavin.[4], [5]

4. *Pyridoxine (vitamin B_6) and fat*

A close relationship exists between the requirement for fat and for pyridoxine.[4], [5] As has already been mentioned, the skin symptoms which originate when essential fatty acids are absent from the diet are similar to those which have been noted in pyridoxine deficiency. BIRCH and GYÖRGY[4] have shown that the dermatitis which occurs on a diet low in pyridoxine but containing 10% of fat can be cured when small amounts of lard are added. In fact, somewhat later BIRCH[146] was able to show that the addition of fat to the diet delayed the onset of dermatitis produced by a pyridoxine deficiency. In some cases, it completely prevented the skin condition, even up to the time of death. SALMON,[147] likewise, demonstrated that vitamin B_6 and fats supplement each other in curing dermatitis. Most of the evidence indicates that the effectiveness of the fat is directly proportional to its degree of unsaturation and thus

presumably to the essential fatty acid content. The latter explanation might readily account for the fact that lard, which ordinarily has a relatively high essential fatty acid content, will be effective in alleviating pyridoxine deficiency, whereas butter, which has a relatively low essential fatty acid content, is somewhat less efficacious. SCHNEIDER[148] attributed the low anti-dermatitis potency of rancid butter to the destruction of its linoleic acid.

A number of workers have demonstrated that all commonly occurring essential acids are effective in curing pyridoxine deficiency. Of these several acids, linoleic acid appears to be the most effective,[149] whereas both linolenic acid and arachidonic acid have been shown to be less satisfactory in clearing up the dermatitis. SALMON,[147] and SCHNEIDER and co-workers,[150] agree that linoleic acid possesses a greater potency in this respect than does linolenic acid. They point out that the effectiveness of several natural fats in clearing up the acrodynia is in proportion to their linoleate content. Corn oil was shown to have a greater activity than linseed oil; other fats exerted a curative effect proportional to their linoleate content, while cod-liver oil was completely ineffective. According to RICHARDSON *et al.*,[151] methyl arachidonate and linoleic acid were useful in clearing up the dermatitis caused by pyridoxine deficiency, but did not afford permanent protection. The latter workers suggest that the unsaturated acids do not replace pyridoxine but simply delay the onset of the skin symptoms.

SARMA and associates[152] have shown that the growth inhibition in rats caused by feeding diets containing suboptimal amounts of pyridoxal or pyridoxine is accentuated when oleic acid is added to the ration. However, this inhibitory effect of oleate can be counteracted by the administration of additional quantities of vitamin B_6. Non-essential fatty acids have been shown to increase the deficiency symptoms in the rat. Thus, the administration of hydrogenated coconut oil[13–15] to rats suffering from fat-deficiency symptoms resulted not only in greater loss in weight but also in a more rapid mortality. Elaidin has likewise been shown to accentuate this essential fatty acid deficiency.[16]

The question which naturally arises is whether or not the essential fatty acid and pyridoxine deficiencies are entirely similar. Although MEDES and co-workers[153] emphasized the interrelation between the two types of deficiency, their results might be interpreted to mean that two different factors are involved. Thus, when the diet was lacking in both of these components, a relief from the deficiency was obtained by the administration of either ethyl linoleate or pyridoxine. However, the resulting growth response was less with maximum amounts of ethyl linoleate than it was with optimum doses of pyridoxine. Best results were obtained when both of these components were included in the diet simultaneously.

One possible explanation of the importance of pyridoxine in fat metabolism is that this factor is required by the mammal in the synthesis of fat from protein.[154] UMBREIT and GUNSALUS[155] have demonstrated that vitamin B_6 is one of the essential steps in the production of fat from carbohydrate;

the importance of this vitamin in fat synthesis is therefore immediately evident.

5. *Niacin, folic acid, biotin, and fat*

It has long beeen known that both niacin and folic acid are synthesized in the intestine of the rat. ELVEHJEM[135] has shown that the rate of this synthesis is accelerated on a diet containing fat. He reported that butterfat had a greater value than did corn oil.

In addition to its sparing action on thiamin and pyridoxine, fat exerts a similar action on niacin. According to SALMON[82], [147] the requirement for nicotinic acid is lower when fat is the chief source of calories than when the diet is predominantly carbohydrate.

It now appears evident that biotin likewise exerts a pronounced effect on fat metabolism. As early as 1927, BOAS[156] reported that practically no body fat was present in the tissues of rats which had suffered severe biotin deficiency. On the other hand, the administration of this vitamin to rats increases fat synthesis; it will likewise provoke a fatty infiltration of the liver.[157] These fatty livers have a high cholesterol content and resemble those occurring after the feeding of a fraction from beef liver.[154] PAVCEK and SHULL[158] have demonstrated that biotin is readily destroyed by rancid fats, but that tocopherol is an excellent protective agent to prevent this destruction. It has been suggested that biotin is changed to biotin sulphoxide when this inactivation takes place.[159] The resulting biotin compound no longer supports the growth of *Lactobacillus casei*, although it can still stimulate the growth of yeast.[158] Biotin plays a major role in the growth of micro-organisms. In most cases, the requirement varies with the amount of oleic acid or other unsaturated acids present in the medium. This subject is discussed in a later section (see p. 137).

6. *Fat-soluble vitamins and fat*

Since fats are excellent media for the solution and stabilization of the fat-soluble vitamins, it is obvious that the quality and quantity of fat in the diet are of prime importance in the absorption of these vitamins from the gastro-intestinal tract.

In the case of vitamin A, MUELDER and KELLY[160] have shown that the absorption of the vitamin by depleted rats was aided by dietary fat, although its subsequent utilization was independent of the level of this foodstuff. On the other hand, it was shown by RUSSELL and collaborators[161] that the utilization of vitamin A in the chicken was not related to the fat level of the diet, although the absorption of carotene was dependent upon the presence of this substance.

Although, under most conditions, preformed vitamin A has been considered to have the same biological value when administered as the ester or as the free alcohol,[162] WEEK and SEVIGNE[163–165] have reported a number of instances in which this was not the case. Thus, when difficultly absorbed oils or waxes were employed as the diluents, vitamin A was invariably best utilized when given in the form of the free alcohol. When vitamin A deposition in the livers of chicks

was used as the criterion of vitamin A utilization,[163] the results were practically identical when cottonseed, corn, sardine, or castor oil was the diluent, irrespective of whether vitamin A was fed as the alcohol or as the acetate. On the other hand, when the solvent was basking shark oil, sardine oil, jojoba seed oil, ethyl laurate, or mineral oil, the acetate was considerably less readily utilized than was the free alcohol. In all cases, the natural vitamin A ester was definitely inferior to the other two forms of vitamin A employed in these tests in causing vitamin A deposition in the liver of chicks. Table 11 gives a summary of these interesting results.

Table 11. The average storage of vitamin A in the livers of vitamin A-depleted chicks after the feeding of 30,000 units of vitamin A in the form of alcohol, acetate, or natural ester, in three divided doses with several diluent oils[163]

*Diluent oil**	*Series No.†*	*Vitamin A per liver after*			*Vitamin A stored after*		
		Alcohol	*Acetate*	*Natural ester*	*Alcohol*	*Acetate*	*Natural ester*
Vitamin A fed in 3 doses of 2 ml each with concentration of 5000 units/ml							
		units	*units*	*units*	%	%	%
Basking shark . .	I	4510	2390	1880	15·0	8·0	6·3
Castor . . .	I	8210	8020	5090	27·4	26·7	17·0
Corn . . .	I	7150	5970	4940	23·8	19·9	16·5
Corn . . .	II	10670	10080	7130	35·6	33·6	22·8
Cottonseed . .	I	7490	6830	5320	25·0	22·8	17·7
Ethyl laurate . .	II	8830	4240	3660	29·4	14·1	12·2
Jojoba seed . .	II	9850	4770	2440	32·8	15·9	8·1
Mineral oil . .	I	4860	2160	1465	16·2	7·2	4·9
Sardine oil . .	I	3930	3960	1390	13·1	13·2	4·6
Vitamin A fed in 3 doses of 0·1 ml each with concentration of 100,000 units/ml							
Corn . . .	II	10380	11690	8320	34·6	39·0	27·7
Jojoba seed . .	II	9170	10490	8400	30·5	35·0	28·0

* Control tests on chicks given each diluent oil without vitamin A yielded negative results for vitamin A in the liver in all cases.

† Series I, New Hampshire chicks, 52 days old, 10 chicks per group; Series II, white Leghorn cockerels, 56 days old, 12 chicks per group.

A somewhat similar situation obtains in the rat, as was shown by later tests of WEEK and SEVIGNE.[164] Only 0·4 ml doses of the solutions were employed in this case, as compared with 2·0 ml in the chick tests. Although no differences in the degree of utilization of vitamin A were noted when it was given as the free alcohol or as the acetate, respectively, a markedly inferior response occurred when the vitamin A was administered as the natural ester in jojoba oil. Finally, in unpublished experiments of the author,[166] it was found that vitamin-depleted rats exhibited less growth, during a 28-day bioassay period, when vitamin A was administered as the natural ester in jojoba oil than when vitamin A alcohol was used with this solvent. In cases in which cottonseed oil was

employed as the diluent, no differences in growth response were observed when different forms of the vitamin were employed.

It has recently been demonstrated that the action of vitamin A may be conditioned by the form in which it is administered, in the case of human subjects. WEEK and SEVIGNE[165] concluded, on the basis of vitamin A tolerance curves in the blood, that the rate of absorption of vitamin A by male subjects, after 134,000 μg of vitamin A had been given in 50 gm of margarine, was best with the alcohol, somewhat less satisfactory with the acetate, and poorest with a mixture of natural esters. The experiments with female subjects showed that the vitamin A alcohol was significantly better than the acetate but not superior to the natural ester.

The utilization of β-carotene by the rat depends upon the solvent in which it is fed.[167], [168] FRAPS and MEINKE,[169] using a liver storage test, found that carotene was less readily utilized when present in vegetables than when fed as a component of cottonseed oil. KREULA and VIRTANEN[170] found that, in the case of human subjects, the absorption of carotene from carrots was less efficient than that from butterfat. RUSSELL *et al.*[161] reported that β-carotene is efficiently utilized by the chick only if fat is also present. According to BURNS *et al.*,[171] both carotene and vitamin A were more effective in the rat when fed with a diet containing 5% of lard than when given with a fat-free diet which was supplemented with 0·1 gm of corn oil daily. The utilization of carotene by rats has been shown to be improved by incorporating it in margarine.[172] It is suggested that this latter effect is potentiated by the emulsifying agents present in this product.

It has long been known that the presence of mineral oil in the diet has a deleterious effect on the utilization of some of the fat-soluble vitamins. Some interference may obtain in the utilization of vitamin A,[173] while the depressing effect on the absorption of carotene is greater.[174] BURNS and associates[175] have noted a decreased utilization of carotene when as small an amount as 0·08% of mineral oil was present; some effect on vitamin A utilization obtained when the level of mineral oil in the diet was increased to 0·16%. When 0·32% of mineral oil was incorporated in the diet, the utilization of vitamin A and carotene could not be improved by increasing the level of dietary fat from 5 to 10%. It has also been reported that the utilization of vitamins D and K is impaired by the presence of petrolatum in the gastrointestinal tract.[176] BACON and collaborators[177] have noted that a retardation in growth obtains within 2 to 3 weeks when 10% mineral oil is included in a fat-free ration. It is suggested that this effect is related to the development of an essential fatty acid deficiency.

The hydroxy fatty acids may also have a depressing effect on rats. Thus, NIGHTINGALE *et al.*[177a] reported avitaminosis K in rats when dihydroxystearic acid triglyceride was incorporated in the diet to the extent of 25%. It is possible that the hydroxy acid may interfere with the synthesis of vitamin K which normally occurs in the intestine.

No adequate answer is as yet available to explain the antirachitic effect of

fats on rats receiving high-calcium, low-phosphorus, rachitogenic diets, as reported by BOOTH, HENRY, and KON.[178] These workers found that vegetable fats devoid of vitamin D and triglycerides resynthesized from the fatty acids of butter and peanut oil exerted an antirachitic effect differing from that of vitamin D. It is possible that a differential effect of the several fats on calcium excretion via the intestine, as demonstrated by CHENG and her co-workers,[119] might offer an explanation for this phenomenon.

8. The Effect of Dietary Fat on Galactose Absorption

SCHANTZ, ELVEHJEM, and HART[179] first called attention to the fact that a galactosuria occurred in rats and other animals when they were fed on a liquid skimmed milk diet. On the other hand, it was shown that the excretion of galactose did not occur when a whole milk diet was fed. Moreover, when 3 or 4% of fat was added to the skimmed milk, the galactosuria was completely prevented. It was suggested that fat may play a role in the metabolism of lactose and galactose other than by altering its rate of absorption. In a later report, SCHANTZ and KREWSON[180] reported that several synthetic even-chain fatty acids containing 12 or more carbon atoms were equally effective in preventing galactose excretion in the urine. On the other hand, ZIALCITA and MITCHELL[181] failed to confirm the reported effect of fat, as such, on the metabolism of galactose. They likewise suggest that there is no reasonable deduction, based upon the available facts of animal nutrition, that such an effect would be expected. Corn oil, but not butterfat, was found to be able to decrease galactosuria by about 25%, on a diet containing 48% of lactose. These workers suggest the possibility that this effect may be due to some non-glyceride component in the corn oil. In a later communication of the Wisconsin group, GEYER *et al.*[182] extended the earlier work, employing skim milk and synthetic diets containing lactose or galactose. It was concluded that fat decreased galactosuria, and hence increased the utilization of galactose. These conflicting viewpoints of the several observers leave the question open as to whether the fat effect on galactosuria originates primarily in the absorptive or in the metabolic phase.

The results of NIEFT and DEUEL[183] throw some further light on the problem. Their data strongly support the concept that the absorption mechanism is primarily involved. It is suggested that the delayed evacuation of the stomach caused by the presence of fat might slow down the rate at which lactose (or galactose) reaches the intestine, and hence might result in a lower galactosemia, with a consequent increase in retention. The effect was shown to be unrelated to an upset in the rate of hydrolysis of lactose. Moreover, at a 20% fat level, the relative effectiveness of butterfat, cottonseed oil, and corn oil in reducing the loss of galactose in the urine was found to be identical. Although favouring the theory that fat acts in the absorptive phase of galactose metabolism, NIEFT and DEUEL[183] leave the question open as to whether or not the metabolic phase is involved, especially since the latter hypothesis might be an explanation for the greater ketolytic effect of galactose as compared with glucose, reported for rats[184] and for man.[185]

9. Fatty Acids as Required Growth Factors for Bacteria

1. *Oleic acid as a substitute for biotin*

There is considerable evidence, based upon microbiological studies, that the metabolism of biotin and that of fat are related. On a fatty acid-free medium, the growth of most bacteria requires the presence of biotin. However, if oleic acid is supplied, normal growth may obtain, even in the complete absence of biotin.

COHEN and co-workers[186] were the first to report that oleic acid is required by bacteria. These workers showed that this unsaturated acid is essential for *Corynebacterium diphtheriae*. It was subsequently demonstrated to be a prerequisite for the growth of *Clostridium tetani* and *Clostridium welchii*. WILLIAMS, BROQUIST, and SNELL[187] reported, in 1947, that cultures of several types of lactic acid bacteria required oleic or linoleic acids, or a combination of both, while the even-numbered saturated acids from C_6 to C_{18} were completely inactive. In the case of *Lactobacillus bulgaricus*, although oleic acid was found to be essential for growth, its growth-promoting action could be observed only within a narrow range of concentration and within certain *p*H limits. A particular strain of *Lactobacillus* isolated from the caecal contents of rats was also shown to require oleic acid for growth.[188] This organism could be repeatedly transferred on synthetic media only when oleic acid was present.

WILLIAMS and FIEGER[189] were the first to call attention to the similarity in the stimulating action of oleic acid and of biotin on *Lactobacillus casei*. Although a maximum growth (as determined from acid production) could be demonstrated in the presence of lipids, no synthesis of biotin occurred.[190] It was suggested that the bacteria adapt themselves to either the biotin-free or the biotin-low environment. These results render it doubtful that the fatty acids are concerned with the biosynthesis of biotin in the intestine. This agrees with the data of OPPEL,[191] who reported a relatively constant excretion of biotin in the urine on biotin-free diets, while the sudden variations were related to biotin changes in the diet.

Although *Lactobacillus arabinosus*, *L. casei*, and *Streptococcus faecalis* can grow in the complete absence of biotin, aspartic acid as well as unsaturated acids must be present in the media.[192] On the other hand, BROQUIST and SNELL[192] noted that unsaturated acids alone were necessary to insure growth in *Lactobacillus fermenti* and *Clostridium butyricum*. The latter organism was shown to synthesize aspartic acid when grown in the presence of oleic acid and in the absence of biotin.

It is now believed that the biotin-like component in acid-hydrolyzed plasma is an unsaturated acid. Although TRAGER[193] believed that this ether-soluble substance was a component of the non-saponifiable residue, HOFMANN and AXELROD[194] were unable to confirm this hypothesis. The latter workers reported that the biotin-like substance in beef and human plasma occurred exclusively in the saponifiable portion of the lipid. When the fraction was esterified with diazomethane, the resulting methyl esters obtained by distillation

possessed high biological activity. On a weight basis, oleic acid was shown to have about the same potency as the plasma distillate. It was also found that the liquid acid fraction of the plasma, which contains oleic, linoleic, and arachidonic acids, had a greater growth activity for the *Lactobacilli* than did the original fraction, although the saturated acids were also found to have some synergistic action.[195]

There are a number of hypotheses as to the reason why biotin and unsaturated fatty acids can replace each other as requirements for bacterial growth. TRAGER[196] concluded that biotin functions in the synthesis of fatty compounds or of substances containing fatty acids, such as lecithin. RUBIN and SCHEINER[197] demonstrated that biotin is primarily concerned with the synthesis of one or more fatty acids. Thus, the latter investigators were able to show that such biotin antagonists as homobiotin, trishomobiotin, homobiotin sulphone, and trishomobiotin sulphone have no depressing effect on the growth of *Lactobacillus casei* on a biotin-free medium, provided oleic acid is used as a biotin substitute. In addition, WILLIAMS and FIEGER[198] noted that avidin, sufficient to combine with 1000 μg of biotin, did not prevent lipid stimulation of *L. casei* in the absence of biotin. This would indicate that oleic acid is not active by virtue of effecting a synthesis of biotin. It is therefore apparent that biotin normally functions in bacterial metabolism to bring about the synthesis of oleic acid; biotin antagonists can have no inhibiting action on growth when the products, ordinarily synthesized by the aid of biotin, are furnished in adequate amounts.

Although oleic acid functions as a substitute for biotin with many bacteria in *in vitro* tests, it does not behave in the same manner *in vivo*. For example, TRAGER[199] found that oleic acid injected intramuscularly in chicks did not have the same effect in reducing biotin deficiency produced by the ingestion of avidin (raw egg white) as has biotin or the fat-soluble fraction from hydrolyzed plasma. Moreover, WILLIAMS and FIEGER[200] demonstrated that the lipids from rice polishings, which have a biotin-like activity in the case of *L. casei* and *L. arabinosus*, will not reduce the biotin-deficiency symptoms in white Leghorn chicks.

2. *Comparative potencies of octadecenoic acids*

A large number of isomers of oleic acid, both geometric and positional, can serve as adjuncts in the growth of bacteria in lieu of oleic acid. In the first place, the *trans*-isomer of oleic acid, elaidic acid, has been found by a number of workers[201–205] to possess approximately the same biopotency as oleic acid, although AXELROD *et al.*[195] had assigned to it a somewhat lower potency, and WILLIAMS and FIEGER[190] have reported that it has a somewhat greater effect than has oleic acid.

The location of the double bond does not markedly influence the activity of the *cis*-octadecenoic acids. Thus, petroselinic acid (6-octadecenoic acid),[202, 203, 205] *cis*-8-octadecenoic acid,[203] vaccenic acid (11-octadecenoic acid),[201, 205] and *cis*-12-octadecenoic acid[205] all had about the same growth-stimulating action as did oleic acid, although the *cis*-8 acid appeared to exert a

somewhat greater effect. However, the *cis*-4-octadecenoic acid has been reported to be inactive, as has also the *trans*-isomer.[201]

In addition to the activity exhibited by the *cis*-octadecenoic acids, the *trans* forms are, in many cases, equally potent, although marked variations have been noted. As reported earlier, elaidic acid is equally as potent as oleic acid. However, CHENG and associates[205] found that elaidic acid possessed the maximum potency of the *trans*-isomers tested; as the double bond shifted in either direction from the 9 position, a reduction in potency obtained, until at the 6 position the acids were completely inactive. This was likewise true of 17-octadecenoic acid, which forms no geometric isomers. These results are summarized in Table 12.

Table 12. Biotin-like effect of octadecenoic acids and linoleic acid, alone and with biotin, using Lactobacillus arabinosus[205]

Compound	*cis*-Acid			*trans*-Acid		
	Melting point °C	*Biotin equivalent (mμgm) per mg fatty acid*		*Melting point* °C	*Biotin equivalent (mμgm) per mg fatty acid*	
		Alone	*With biotin*		*Alone*	*With biotin*
6-Octadecenoic acid	29·8	11·6	12·4	53·0	0·4	3·7
7-Octadecenoic acid	—	—	—	43·5–44·5	2·7	15·2
8-Octadecenoic acid	22·7–23·8	14·7	13·4	51·5–52·3	2·8	9·1
9-Octadecenoic acid	13·0	10·3	10·2	44·5–45·5	11·5	10·4
	—	—	—	41·5–42·5	9·6	9·8
10-Octadecenoic acid	—	—	—	52·0–52·6	6·1	11·0
11-Octadecenoic acid	13·0–14·0	12·0	11·4	43·5–44·5	2·6	7·3
	—	—	—	39·0	1·5	7·2
12-Octadecenoic acid	26·8–27·6	10·8	13·4	52·0–53·0	2·7	15·2
17-Octadecenoic acid*	55·5–56·1	0·0	1·8	—	—	—
cis-9, 10-, *cis*-12, 13-Octadecadienoic acid (Linoleic)	–5	5·3	6·9	—	—	—

* No geometric isomers of this compound. Included in *cis* column for convenience.

3. *Comparative potency of other unsaturated acids and related compounds*

AXELROD *et al.*[195] have found that a number of compounds related to oleic acid are likewise effective stimulants for the growth of *Lactobacillus arabinosus*. The following activities expressed in micrograms of biotin per milligram of fatty acid were obtained: oleic acid, 6·0; methyl oleate, 3·2; oleic acid amide, 6·3; oleyl alcohol, $< 0{\cdot}05$; elaidic acid, 1·0; vaccenic acid, 1·2; dihydroxystearic acid (94°C), $< 0{\cdot}05$; and dihydroxystearic acid (130·7°C), $< 0{\cdot}05$.

Linoleic acid (9,12-octadecadienoic acid) possesses a high biotin-like activity,[192] but it is somewhat lower than that of oleic.[195], [202], [203], [205] Linolenic acid (9,12,15-octadecatrienoic acid) is likewise effective in stimulating bacterial growth,[192], [195], [202], [203], [206] but its potency is only a fraction of that of oleic

acid.[195] Azelaic acid ($HOOC(CH_2)_7COOH$) and pelargonic acid ($CH_3(CH_2)_7COOH$), which are oxidation products of oleic acid, are completely inactive.

Activity in substituting for biotin obtains with unsaturated fatty acids other than those having 18 carbon atoms. Thus, palmitoleic acid (9-hexadecenoic acid) was shown by HASSINEN *et al.*[207] to stimulate acid production in the case of *L. arabinosus* in a manner similar to that of oleic acid. When *Lactobacillus bifidus* was used, this acid was likewise able to stimulate growth when present in a low concentration. However, at a higher concentration, palmitoleic acid brought about a growth inhibition of these bacteria, in contrast to oleic acid, which accelerates growth at both low and high concentrations. BOUGHTON and POLLOCK alone[201] and with HOWARD[202], [203] likewise reported a positive stimulation of the diphtheria bacilli on the part of *cis-* and *trans*-palmitoleic acids as well as by the odd-carbon acids, *cis-* and *trans*-heptadec-9-enoic acids.

On the other hand, a number of unsaturated acids are inactive. Thus, BOUGHTON and POLLOCK[201] reported negative results with the following: *cis*-undec-9-enoic acid; *cis*-undec-10-enoic acid; *cis-* and *trans*-myristoleic acid (9-tetradecenoic acid); and *cis-* and *trans*-gadoleic acid (9-eicosenoic acid). Erucic acid (*cis*-13-docosenoic acid) and brassidic acid (*trans*-13-docosenoic acid) were reported to be inactive, as were also α-, β-hydroxyoleic acid and ricinoleic acid (12-hydroxy-9-octadecenoic acid).[203]

4. *The effect of saturated acids on bacterial growth*

In contradistinction to the activity of a number of unsaturated fatty acids tested as substitutes for biotin as a growth factor in bacterial cultures, the saturated fatty acids have been found to be completely inactive when supplied alone. This is true not only of the various even-carbon acids from C_8 to C_{16},[187] but also of stearic acid (octadecenoic acid).[195] Lauric and myristic acids have been reported by WILLIAMS and FIEGER,[190] also, to be inhibitory.

HASSINEN and colleagues[208] noted that growth of both Gram-positive and Gram-negative organisms was inhibited by C_8 and C_{10} saturated fatty acids, but that no significant depressing action was found on the part of C_4, C_6, C_{16}, and C_{18} saturated acids. On the other hand, the C_{12} saturated acid inhibited only the Gram-positive organisms, while some inhibiting action likewise obtained in the case of the C_{14} acid.

On the other hand, the results of CHENG and co-workers[205] indicate that the saturated acids (principally palmitic and stearic acids) have a synergistic action on bacterial growth when present along with oleic acid. This effect is discussed below.

5. *Growth stimulation of bacteria, produced by mixed fatty acids from various natural and artificial fats*

In the study of the biotin-like activities of the mixed fatty acids prepared from a number of fats, it was found that their activities could not be correlated with the content of oleic and linoleic acids unless account was taken of the synergistic action of the saturated acids. The following formula, which takes into account

the variation in activity of oleic and linoleic acids and corrects for the synergistic action of the saturated acids, was found to give excellent results in predicting the activity of the fatty acid mixtures.

Th. s, the biotin equivalent, as micrograms of biotin per milligram of fatty acids, equals:

$$\frac{(\%\ \text{oleic} \times 10{\cdot}3) + (\%\ \text{linoleic} \times 5{\cdot}3)}{100} \times \left(1{\cdot}00 + 2{\cdot}3\ \frac{\%\ \text{saturated fatty acids}}{100}\right)$$

By the use of this formula, the calculated biotin-like effect of mixed fatty acids can be estimated. A comparison of the estimated and found values of fatty acids from several fats is given in Table 13.

6. *Effects of unsaturated fatty acids on growth of bacteria other than the biotin-like effect*

In the extensive studies of KODICEK,[209] it has been shown that most fatty acids possess an inhibitory effect on the growth of various Gram-positive organisms. This behaviour applies to the saturated and unsaturated acids alike. Lauric and myristic acids inhibit bacterial growth in concentrations of 1 : 100,000. In the case of the unsaturated acids, the *cis*-forms have a marked retarding effect on growth, and an increased effect is noted in the case of polyunsaturated acids. Thus, toxicity occurs in the following order: oleic acid < linoleic acid < linolenic acid.

The bacteriostatic action of the unsaturated acids was shown to be reversed by surface-active agents such as lecithin, sterols, tocopherols, proteins, and similar compounds. On the other hand, esters of the fatty acids were not bacteriostatic. In contradistinction to the reversal of the inhibitory effect caused by the surface-active agents on the unsaturated acids, these agents were unable to reactivate the saturated acids.

10. Fatty Acids as Required Growth Factors for Lower Organisms

TRAGER[196] found that oleic acid can replace biotin in the growth of the larvae of the yellow fever mosquito, *Aedes aegypti*. In the absence of biotin, larval growth was very slow, and metamorphosis did not occur. When biotin was present to the extent of 50 mμ mg per ml of medium, excellent growth was obtained. Relatively low concentrations of oleic acid, an oil from hydrolyzed plasma, lecithin, and related compounds, when used in place of biotin, supported larval growth comparable with that obtained with the lower effective doses of biotin. On the other hand, oleic acid and Tween 80, either alone or with aspartic acid, produced some growth response on the part of *cholineless Neurospora crassa* (red bread mould), although they were unable to replace biotin completely.[210]

FRAENKEL and BLEWETT[211] reported that linoleic acid and α-tocopherol are required by the Mediterranean flour moth, *Ephestia kuehniella*. Linoleic acid is needed for the normal development of the wings and for successful emergence, while tocopherol is necessary as an antioxidant for linoleic acid, and to promote

Table 13. Biotin-like activity of fatty acids from several fats and oils as found by microbiological assay and by calculation from fatty acid composition[205]

Fatty acids from:	Composition of total mixed fatty acids			Biotin equivalents of mixed fatty acids		Synergistic effect of saturated acids		Biotin equivalents of mixed fatty acids	
	Saturated (1)	Oleic (2)	Linoleic (3)	Found (4)	Anticipated from (2) + (3) (5)	Increase* (6)	Ratio (6) : (1)	Calculated† (7)	Deviation‡
	%	%	%	mμ gm/mg fatty acid	mμ gm/mg fatty acid	%		mμ gm/mg fatty acid	%
Coconut oil	92	6	2	2·0	0·7	186	2·0	2·2	− 9
Cottonseed oil	26	22	52	9·2	5·0	84	3·2	8·0	+ 15
Olive oil	12	81	7	12·0	8·7	38	3·2	11·2	+ 7
Soybean oil	13	25	62§	7·7	5·5	40	3·1	7·2	+ 7
Butterfat	59	37	3	9·1	4·0	127	2·2	9·4	− 3
Margarine fat	21	74	5	11·8	7·8	51	2·4	11·7	+ 1
Shortening C	38	55	7	8·2	6·1	34	0·9	11·3	− 27
Shortening D	32	66	2	10·6	6·9	54	1·7	12·0	− 12

* ((4) − (5)/(5)) × 100.
† Based upon the composition of the fatty acid mixture, using the formula given above.
‡ ((4) − (7)/(7)) × 100.
§ Including 7% linolenic acid.

rapid growth. On the other hand, arachidonic acid has no effect on wings or on emergence, but it does have a striking effect on growth.[212] Its effect closely resembles that of docosahexenoic acid from cod-liver oil. On the basis of the linoleate composition of body oil, it is concluded that *Ephestia*, which requires linoleate, does not synthesize this acid. On the other hand, another insect which does not require linoleate, namely the meal beetle, *Tenebrio*, is apparently able to synthesize this dienoic acid.[213]

III. The Effect of Dietary Fat on the Response to Stress Factors

There is considerable support for the thesis that the presence of fat in the diet may exert a beneficial effect in times of physiological stress. Thus, during periods of growth, pregnancy, and lactation, animals in general responded better when fat was incorporated in the diet. Moreover, when rats were subjected to complete inanition until they died, it was found that the most prolonged survival followed prefeeding the animals with fat. When the diet during the pre-fast period was carbohydrate or protein, the survival time was curtailed. Moreover, when rats were subjected to physical exertion to the point of complete exhaustion, a greater physical capacity was noted in the rats receiving a high-fat diet than in those on a fat-low regimen. These phases of fat nutrition have been developed in Section II.

It is likewise of considerable interest to observe how diet will modify the response of animals to *external* forms of stress. Thus, the present discussion will centre around the question as to whether or not dietary fat can modify the normal response to thyrotoxicosis, x-radiation, cold environmental temperatures, and similar forms of stress.

1. Thyrotoxicosis and Dietary Fat

When an excess of the purified thyroid hormone or desiccated thyroid powder is given to rats and to other animals, as well as when the thyroid gland becomes sufficiently hyperactive, certain readily reproducible symptoms develop. Suppression of growth normally occurs concomitantly with an increased basal metabolism. The heart rate is increased proportionally with the rise in the basal metabolic rate. Inhibition in ovarian development occurs in young rats. On the other hand, the kidneys, adrenal glands, and heart become considerably hypertrophied.

The beneficial effect of the inclusion of fat in the diet of hyperthyroid animals was first noted by Abelin and his co-workers.[214–216] These workers reported that a high-fat diet was able to counteract the increased metabolic rate, as well as to prevent the reduction in liver glycogen which follows the administration of the thyroid hormone. A number of workers[217–219] have confirmed Abelin's work on rats, while Berg[220] has corroborated the protective action of fat in dogs, manifested by a reduction in the basal metabolic rate following hyperthyroidism.

One explanation for the protective action of fats in thyrotoxicosis is that they furnish the necessary essential acids. However, not only linoleic acid, but also

the non-essential oleic acid, was shown to prevent the rise in metabolism.[221] On the other hand, GUERRA[222] correlated the protective action of fat with its unsaturation. According to ZAIN,[223], [224] linoleic acid but not stearic acid prevents the loss of liver glycogen after massive doses of thyroid extracts. Oleic acid was shown to occupy an intermediate position. It is of course possible that the negative effect of stearic acid in affording protection against thyrotoxicosis in the above studies may have been due to the failure of the saturated acid to be absorbed.

The recent reports of ERSHOFF[225] as well as of GREENBERG and DEUEL[226] have shown that the beneficial effect of fat in preventing growth inhibition of rats is rather generalized. Thus olive oil, cottonseed oil, peanut oil, corn oil, soybean oil, hydrogenated cottonseed oil, wheat germ oil and lard, all caused a significantly greater growth of rats than obtained on the fat-low ration, irrespective of the essential fatty acid content of the various substances.[225] However, in these tests fat failed to prevent inhibition of ovarian development, or the enlargement of the kidneys, adrenal glands, and heart. In the experiments of GREENBERG and DEUEL,[226] not only did the presence of fat permit normal growth in the presence of desiccated thyroid powder, but also it reduced mortality to 0. Table 14 gives a summary of the latter results.

Table 14. The mean weights and mortality of rats on low-fat and high-fat diets without or with thyroid powder[226]

Basal diet	*Desiccated thyroid present*	*Body weight*			*Gain in weight*	*Mortality*
		Start	*After 6 weeks**	*After 11 weeks*		
Male rats						
		gm	gm	gm	gm	%
Low-fat	0	41·1	173·3 ± 4·0	203·3	162·2	0
Low-fat	+	43·7	138·7 ± 2·4	141·7	98·0	50
Low-fat†	+	44·0	114·0 ± 3·1	134·0	90·0	83
High-fat	0	41·8	207·0 ± 14·9	271·7	229·9	0
High-fat	+	43·8	216·3 ± 7·5	280·2	236·4	0
Female rats						
Low-fat	0	46·3	134·7 ± 7·4	161·0	114·7	0
Low-fat	+	45·3	135·2 ± 4·1	121·0	75·7	50
Low-fat†	+	43·8	117·5 ± 10·5	135·0	91·2	83
High-fat	0	44·7	166·5 ± 3·9	200·7	156·0	0
High-fat	+	43·8	175·8 ± 7·4	208·3	164·5	0

* Including Standard Error of the Mean.
† Group in which the level of B vitamins was twice that in other groups.

The reason for the protective action of fat on thyrotoxicosis has not been established. ERSHOFF[225] suggested the possibility that the fat may furnish essential fatty acids and thus alleviate a deficiency which may arise as a result

of an increased fatty acid requirement. However, since a practically fat-free liver preparation was also shown to have a beneficial effect, ERSHOFF does not believe that the fatty acid deficiency theory is a plausible one. On the other hand GREENBERG[227] was able to demonstrate a protective action against thyrotoxicosis in the rat on the part of both methyl linoleate and cottonseed oil. It has also been suggested that the beneficial effect of fat in this condition may result from synthesis of an antithyrotoxic factor by the intestinal flora.[227] According to TEMPLETON and ERSHOFF,[113] both fat and carbohydrate are superior to protein (casein) in permitting survival of rats receiving thyroxine.

2. X-Radiation Injury and Dietary Fat

DECKER and associates[228] were the first to show that a fat-deficiency syndrome can be precipitated in mice by the application of x-radiation. Mice receiving fat were found to be more resistant to injury from x-radiation than were animals on a fat-free regimen. In an extension of this work, CHENG *et al.*[229] have confirmed the results on rats; moreover, it was found that, in young rats, only the male animals were protected by fat while, in the case of the older animals, fat feeding produced beneficial effects equally in both sexes. The protection afforded by fat was exhibited equally well in groups of rats receiving diets containing 2% of cottonseed oil and in those in which 15% or 30% of fat was present. In later work,[230] methyl linoleate in doses as low as 10 mg was shown to be efficacious as a prophylactic agent, although this dosage of linoleate is far below the optimum level for growth. The results of these investigations are summarized in Table 15.

Table 15. The average length of survival and average exposure to x-radiation of old and of young rats on different diets and subjected to repeated sublethal doses of x-radiation[229]

Series, sex, and diet	*Average day of death**		*Average exposure per rat in r**	
	Male rats	*Female rats*	*Male rats*	*Female rats*
Series I (18-month rats):				
Diet 1 (fat-free)	34·3 ± 3·3	39 ± 4·3	1235 ± 54	1270 ± 77
Diet 2 (15% cottonseed oil)	70·6 ± 6·1	76 ± 7·0	1860 ± 112	1950 ± 128
	5·22	**4·52**	**5·08**	**4·55**
Diet 3 (30% cottonseed oil)	63·7 ± 7·1	69 ± 5·3	1710 ± 119	1800 ± 111
	3·61	**4·42**	**3·63**	**3·92**
Series II (3-month rats):				
Diet 1 (fat-free)	51 ± 3·1	61 ± 3·6	1770 ± 99	1980 ± 84
Diet 2 (2% cottonseed oil)	65 ± 3·0	67 ± 2·6	2205 ± 92	2170 ± 62
	3·25	**1·35**	**3·22**	**1·82**
Diet 3 (30% cottonseed oil)	67 ± 3·2	62 ± 3·4	2290 ± 82	2080 ± 87
	3·60	**0·20**	**4·07**	**0·82**

* Including the Standard Error of the Mean. The bold face figures are the ratios of Mean Difference and $\sqrt{(\text{S.E.M.})^2_{\text{control}} + (\text{S.E.M.})^2_{\text{exp.}}}$. When this figure exceeds 3·0, statistical significance is indicated.

3. Cold Stress and Dietary Fat

It has been known for many years that metabolism is greatly increased by cold. As early as 1881, KÜLZ[231] demonstrated that the liver glycogen of a dog disappeared almost completely if the animal was exposed to prolonged and severe chilling. In studies on human subjects exposed to intense cold, MITCHELL and co-workers[232] found that a high-fat diet is superior to a high-carbohydrate regimen in maintaining general psychomotor performance and visual efficiency, as well as in allowing a maximum speed of tapping. Superiority of the high-fat meals over high-carbohydrate rations in maintaining tissue temperatures in a cold environment appears to be related to a decrease in heat emission rather than to any resulting increase in heat production. A temporary deposition of dietary fat in the subdermal tissues following a fat meal may be related to this phenomenon. However, the fat diet was not appreciably more effective in preventing the cooling of the skin. Likewise, 200% of the basal calories were required for the maintenance of body weight on the high-fat diet, as contrasted with a figure of 188% for the high-carbohydrate diet.

4. Hepatectomy and Dietary Fat

One of the most critical forms of stress which can be applied is that resulting from partial removal of the liver. This is because the liver is important in so many physiological functions which it is impossible or difficult to delegate to other organs. Thus, it serves as a storehouse for glycogen, for the fat-soluble vitamins, as well as for the water-soluble vitamins, and to a certain extent for fats. Moreover, it is the chief site of the formation of ketone bodies, of deamination, of oxidation of uric acid (in dogs and lower animals), of urea synthesis, and of many detoxication reactions. It aids in the utilization of fructose. Moreover, this organ serves as the chief organ for the synthesis of serum albumin and serum globulin. As a result of stimulation by vitamin K, prothrombin is produced in the liver cells. One very important function of this organ is the excretion of such products as bile pigments, cholesterol, and related compounds.

Hepatic tissue is responsible for the mediation of so many vital functions that animals cannot long survive the removal of this organ. However, MANN,[233], [234] many years ago, surmounted the immediate difficulties inherent in the removal of the liver. He was able to maintain life for as long as 11 hr in dogs in which a collateral circulation had been established as the result of a reverse Eck fistula, performed several weeks before the hepatectomy was carried out. MARKOWITZ and SOSKIN[235] were able to develop a similar degree of collateral circulation by a preliminary partial ligation of both the vena cava and the portal vein. Although these procedures extend the life of the animals following the removal of the liver, it has been impossible to alter the fatal outcome by dietary means, except that survival is extended for many hours when the normal blood sugar level is maintained by means of periodic injections of glucose. However, this stimulatory effect of glucose soon loses its value, and the animals no longer recover following its administration.

In spite of the inability of animals to survive the complete removal of the

liver, even under the most satisfactory experimental conditions, rabbits are able to recover if as small an amount as 30% of the functioning tissue of the liver remains.[236] After partial hepatectomy, the rate of regeneration of the liver is extremely rapid. Such experimental conditions as partial hepatectomy therefore provide an ideal stress for the study of the role of the diet.

In the case of rats, BRUES *et al.*[237] reported that regeneration of liver tissue proceeded at a slower rate when fat was included in the diet than when protein or carbohydrate was the chief dietary component. Because of the importance of bile in the utilization of fat, the use of fat diets has generally been avoided in studies of liver regeneration. However, ROGERS and associates[83] have recently reported that moderately high-fat diets are as efficacious in permitting recovery of rats following a 70% partial hepatectomy as are low-fat rations. When rats on the 30% fat diet were limited in food intake to that of the animals on the 3% fat regimen, a significantly greater nitrogen balance and a higher increment in liver protein and in body weight obtained. When the consumption of food was *ad libitum* in both series of tests, the animals on the high-fat diet did considerably better than those on the low-fat intake.

5. Miscellaneous Stress Factors and Dietary Fat

One stress factor which can be counteracted much more effectively by fat than by other foodstuffs is that caused by the administration of 2,4-dinitrotoluene (DNT). CLAYTON and BAUMANN[238] demonstrated that mice and rats grew less and died more rapidly when fed a low-fat diet along with DNT than when fed the same amount of the nitro compound per calorie in a diet containing 5 or 30% of added fats. It was believed that the harmful effects of the low-fat diets were to be ascribed to the low caloric intake of the animals. The beneficial effect of fat in minimizing the toxic action of DNT in mice was demonstrated with cottonseed oil, corn oil, Crisco, hydrogenated coconut oil, lard, and butter-fat. Rancid cottonseed oil was found to augment the toxicity of DNT.

LUNDBAEK and STEVENSON[239] reported that, after hypothalamic injury, the weight gain of the rats suffering from hypothalamic hyperphagia was greater on a high-fat, non-carbohydrate diet than on a high-carbohydrate diet. The weight gain per calorie was likewise shown to be greater when fat was the main foodstuff than when carbohydrate was the principal nutrient. It was suggested that this may be attributed to the fact that the animals on the high-fat diet do not experience a loss of energy entailed by the transformation of carbohydrate into fat.

IV. Factors Altering the Nutritional Value of Fat

1. Sex as a Factor in Fat Utilization and Requirement

Much evidence has developed in recent years indicating that a sex difference obtains not only in the distribution of lipids and glycogen in animal tissues but also in the metabolism of fats. Most recently it has been shown that variations in the essential fatty acid requirement occur between male and female rats.

1. *Sex differences in ketonuria*

Ketonuria is obtained more quickly, and occurs with a higher degree of intensity, in the female than in the male. DEUEL and GULICK[240] were the first to report that the ketone body output based on grams per square metre of body surface per day averaged 5 times as much in women during fasting as in men. A similar sex difference was later demonstrated in rats and guinea pigs when acetoacetic acid, butyric acid, or other ketogenic acids, in the form of sodium salts, were administered to the fasting animals.[241] By means of this procedure, a so-called "exogenous" ketonuria was produced which has proved of value in the investigation of fat metabolism. BEACH and associates[242] have recently recorded a similar sex difference in the ketonuria of rats suffering from alloxan diabetes. Moreover, when rats with fatty livers are subjected to a fast, an "endogenous" ketonuria occurs during the inanition period, even when no supplementary ketogenic acids are fed. Under these conditions, also, the level of ketone bodies excreted is considerably higher in the case of the female rats than in the male animals.[243] The relation of the variation in ketonuria to sex is further suggested by the demonstration that the high ketonuria ordinarily noted in the normal female rat is reduced, following ovariectomy, to less than the value for male animals.[244]

2. *Sex variation in tissue lipids*

DEUEL and associates[245] reported that the total tissue lipids of female rats on several fat diets exceeded those of males on similar regimens, while the tissue content of protein and water was somewhat higher in the males.

The most pronounced variations in lipid composition have been noted, by various investigators, in the liver. Higher levels of liver lipid in the female are invariably associated with a lower glycogen concentration in this organ. A number of investigators have recorded higher liver fat levels in females than in males under a variety of conditions.[243], [246–251] Many workers[243], [246–248], [252], [253] have also reported that liver glycogen is consistently lower in the mature female than in the male. However, a sex difference was not observed in immature or old rats[254] or in female rats following ovariectomy;[254] in fact, in the latter case, the level was slightly higher for the females.

A possible clue to the correlation between the variation in composition of lipids in the tissues and the ketonuria has been observed in rats having fatty livers. Although the liver lipids of unfasted rats on choline-free diets were markedly higher in females than in the males, a greater drop in liver lipids occurred in the female than in the male during a subsequent 5-day fasting period, concomitantly with a higher ketonuria.[255] This was interpreted as indicating that the greater production of ketone bodies in the females was associated with a more rapid removal of the lipid from the liver.[255]

Another expression of sex difference in lipid storage is to be found in the studies of LORENZ, CHAIKOFF, and ENTENMAN.[256] These workers reported that the level of neutral fat was much higher in the livers of laying hens than in the non-laying fowl or in male birds. Although no similar variation in phospholipid

and cholesterol in the liver obtained under these conditions, a significant increase in these components, as well as in neutral fat, was noted in the blood.

3. *Sex differences in requirements for essential fatty acids*

Sex differences in composition and metabolism may be reflected in the requirement for essential lipids. This was recently demonstrated by GREENBERG *et a* .[29] Whereas the optimum level for linoleate intake exceeds 200 mg per day in the male rat,[15] that for females based upon growth response appeared at first to be approximately 10 to 20 mg per day.[28] However, in later experiments, a value of 50 mg was noted for female rats.

Closely associated with the difference in linoleate requirement is the sex difference in susceptibility to x-radiation injury. Whereas, in the case of old rats, equal protection was afforded by fat in the case of both sexes, young adult female rats were not protected by fat in the diet, whereas young adult males were protected.[230] Since it has also been demonstrated that protection from x-radiation injury is afforded by the administration of linoleate,[230] the question arises as to whether or not a sex variation in the amount of this essential acid in the tissues may account for the discrepancy between the sexes.

2. Digestibility and Absorption of Fats as Related to their Nutritional Value

The importance of fat in nutrition is obviously related to its ability to be digested and absorbed. Thus, in order to serve as a source of calories, fat must gain entrance into the tissues before it can be oxidized and yield its potential energy to the tissues. In a like manner, the utilization of fat is necessary before it can serve in its other capacities. It has already been noted that hydrogenated perilla oil and tristearin do not possess the thiamin-sparing action of the other fats, because of their low digestibility.

The extent to which a fat is utilized (i.e. its digestibility) is expressed as the *coefficient of digestibility*. This term refers to the percentage of the foodstuff which is absorbed. This value is calculated by dividing the fat absorbed (fat ingested minus fat excreted) by the fat ingested. A correction figure is usually applied to total faecal fat to correct for the so-called "metabolic fat." This latter fraction is composed of lipid-like material which is excreted irrespective of the amount of fat in the diet; it can readily be determined from the lipid content of the faeces obtained when the subject is on a lipid-free diet.

1. *The digestibility, in man, of vegetable and animal fats melting below 50°C*

Practically all vegetable and animal fats melting at less than 50°C are almost completely digested by the normal individual. The most comprehensive study of this property was made in an extended series of tests carried on by the Office of Home Economics of the U.S. Department of Agriculture under the direction of the late Dr. C. F. LANGWORTHY and of Dr. A. D. HOLMES. In these tests, the fat under consideration was mixed with a fat-free corn starch blancmange and was fed with other foods of low fat content (oranges, whole wheat biscuit, sugar, black coffee) over a 3-day period, during which the faeces were collected for analysis. Although no allowance was made for the faecal fat excreted as soaps

in these tests, more recent data would seem to indicate that this additional correction would not appreciably change the findings in the fats and oils covered in the present category. Thus, DEUEL *et al.*[257] obtained results for the coefficients of digestibility of cottonseed oil and rapeseed oil in human subjects, when account was taken of the soap excretion, which are identical with figures found in earlier tests in which this value was not determined. Another feature of these more recent tests was the use of a new technique involving a much longer experimental period, and the use of an appetizing mixed diet in which the experimental fat comprised 90% of the total fat. Table 16 gives a summary of the results which have been reported for the digestibility of several vegetable and animal fats in man.

According to the results given in Table 16, it would appear that most animal and vegetable fats are well digested by man when taken in amounts of 50 to 100 gm daily. With the exception of avocado fat and teaseed oil, which have coefficients of digestibility of 88 and 91 respectively, the average figures for the 34 vegetable fats varied between 94 and 99, and those for the 18 animal fats ranged between 93 and 99.

2. *The digestibility, in man, of vegetable and animal fats melting above 50°C*

When the melting points of fats exceed a critical value of approximately 50°C, the digestibility is much less than that observed for lower-melting fats. LANGWORTHY and HOLMES[266] called attention to the fact that the high-melting fats could not be assimilated as completely as the low-melting fats, while DEUEL and HOLMES[264] suggested that an inverse relationship exists between the extent of digestibility and the melting point of fat. Data on utilization of some high-melting fats are included in Table 17.

Although the coefficients of digestibility of the several natural high-melting fats (deer, mutton, oleostearin) and of several of the higher-melting hydrogenated fats are definitely lower than those for the vegetable and animal fats reported in Table 16, they are to be regarded as maximum rather than minimum values for human subjects. This is because allowance was not made for the portion excreted in the faeces as soaps which, in this case, would probably represent a fairly large figure. The discrepancies between the reported values for digestibilities in man and in other animals can probably be attributed to this fact. Further experiments are needed to clarify the absolute extent of digestibility of the several higher-melting fats in man.

3. *The digestibility of oleomargarines in man*

Hydrogenation *per se* does not lower the digestibility of the fat unless the resulting mixture has a sufficiently high melting point. Moreover, when completely hydrogenated corn, cottonseed, or peanut oil, melting at above 60°C, is mixed with untreated oil so that the melting point of the resulting blend is under 50°C, the coefficient of digestibility is not appreciably lowered below that of the liquid fats.

In a study of the digestibilities of the older types of oleomargarine obtainable

Table 16. Average coefficients, in man, of digestibility of vegetable and animal fats melting below 50°C

Fat tested	Reference	Average daily fat intake	Coefficient of digestibility
Vegetable fats:		gm	
Almond	258	70	97
Apricot kernel	259	70	98
Avocado	260	100	88
Black walnut	258	56	98
Brazil nut	260	84	96
Butternut	258	46	95
Chariock	261	58	99
Cherry kernel	259	59	98
Cocoa butter	262	51	95
Coconut	262	64	98
Coconut	263	28	89
Cohune	260	52	99
Corn	261	80	97
Cottonseed	262	86	98
Cottonseed	257	60	97
Cupuassu	260	41	94
English walnut	258	78	98
Hempseed	260	54	98
Hickory nut	258	95	99
Japanese mustardseed	261	79	96
Java almond	264	60	97
Melon seed	259	41	98
Olive	262	73	98
Palm kernel	260	100	98
Peach kernel	259	62	97
Peanut	262	98	98
Pecan	258	104	97
Poppyseed	260	50	96
Pumpkinseed	259	75	98
Rapeseed	261	82	99
	257	60	99
Vegetable fats: (contd.)		gm	
Sesame	262	90	98
Soybean	261	82	98
Soybean	263	21	94
Sunflowerseed	261	90	96
Teaseed	264	49	91
Tomato seed	259	57	96
Watermelon seed	264	30	95
Animal fats:			
Beef	265	100	93
Brisket	266	80	97
Butter	265	100	97
Butter	263	26	80
Butter	267	40	97
Chicken	266	95	97
Cod-liver	264	47	98
Egg-yolk	266	83	94
Fish	266	60	95
Goat's butter	268	45	98
Goose	266	95	95
Hard palate	268	90	94
Horse	268	65	94
Kid	268	62	95
Lard	265	90	97
Milk fat	266	78	97
Oleo	268	59	97
Ox marrow	268	87	94
Ox tail	268	77	97
Turtle	268	40	99

Table 17. Average coefficients of digestibility, in man, of some high-melting animal fats and some hydrogenated vegetable fats

Fat tested	*Reference*	*Melting point*	*Average daily fat intake*	*Coefficient of digestibility*
		°C	gm	
Mutton	265	50	53	88
Oleostearin	268	50	68	80
Deer	264	51·4	46	82
Hydrogenated fats:				
Cottonseed	270	35	84	97
		46	89	95
Peanut	270	37	76	98
		39	78	96
		43	91	96
		50	59	92
		52	62	79
Corn	270	33	78	95
		43	74	95
		50	44	88
Blended hydrogenated fats:*				
Cottonseed	264			
(12·5 : 87·5)†		45·8	53	96
(18·8 : 81·2)		47·8	76	94
(23·5 : 76·5)		48·1	49	94
(22·1 : 77·9)		50·0	57	87
Peanut	264			
(6·2 : 93·8)		43·0	74	97
(9·1 : 90·9)		43·2	80	97
(33·3 : 66·7)		51·1	90	93
Corn	264			
(9·1 : 90·9)		39	103	95
(25·0 : 75·0)		49	105	93
(30·8 : 69·2)		54	92	92

* A portion of the fats was almost completely saturated with hydrogen and was blended with sufficient untreated oil to yield mixtures with the melting points indicated.

† The values in parentheses indicate the respective proportions of completely saturated fat and untreated oil in the fat blend.

in 1915, HOLMES[271] reported that they were well utilized. The values for the several types studied were as follows: (i) (59% oleo oil, 7% lard, 22% vegetable oils, 12% milk fat), 97; (ii) (41% oleo oil, 32% lard, 24% peanut oil, 3% milk fat), 93; and (iii) (67% oleo oil, 33% cottonseed oil, 0·1% milk fat),* 97.

A high digestibility for margarine fat was also shown to hold true in the case of a modern type of product in which the fat is exclusively a partially hydrogenated vegetable oil.[267] Thus, this product, which had a Wiley melting point of 94 to 95°F, was found to be digested to the extent of 96·7% when taken by normal

* These are HOLMES' figures, which add up to more than 100%.

men and women in average amounts of 86 gm daily. All of the tests on oleomargarine recorded here are open to the same criticism as are the other experiments on human subjects, namely, that faecal soaps were not determined. Howe er, it seems probable that this would not have influenced the result, inasmuch as a high digestibility was noted for this fat, in rats, in tests which took in consideration the loss of fat in the faeces as soaps (see Table 20). LANGWORTHY[269] has summarized most of these data elsewhere.

4. *The digestibility of fats in animals other than man*

It is generally agreed that the pattern of digestibility of fats is similar in man, dog, and rat. On the other hand, McCAY and PAUL[272] have reported that guinea pigs digest a number of the common fats quite poorly. In later studies, these workers[273] found that rabbits and sheep utilized fat to a somewhat greater extent than did guinea pigs. Table 18 gives the average coefficients of digestibility of various fats as recorded for several species of animals.

Although the coefficients of digestibility in man and in lower animals are in general agreement, a number of striking differences exist. In the first place, castor oil is readily digested in rats, rabbits, sheep, and guinea pigs, and exerts no catharsis in these animals; on the other hand, practically none is utilized in man when sufficient is given to produce a cathartic action. However, it is possible that, when administered in amounts too small to produce catharsis, it might be well utilized in the human subject. In spite of the practically complete digestibility of castor oil in rats, STEWART and SINCLAIR[275] were unable to demonstrate any trace of its principal fatty acid, ricinoleic acid, in the phospholipids of the small intestine, liver, or muscle or in the triglycerides of the liver. This result can only be interpreted as evidence of the rapid metabolism of this hydroxy acid.

A second difference between man and the rat concerns the coefficient of digestibility of rapeseed oil. DEUEL, CHENG, and MOREHOUSE[281] have reported that, in the rat, crude rapeseed oil was utilized to the extent of only 77%, while the figure obtained for the refined oil was 82%. This is the lowest digestibility which has been recorded for a triglyceride fat, liquid at ordinary temperatures, which is not an intestinal irritant. This finding is in line with an earlier observation[283] that the rate of absorption of rapeseed oil from the gastrointestinal tract of the rat is the lowest for any of the common liquid fats. The low digestibility of rapeseed oil in the rat would likewise explain its poor showing in the growth tests reported by BOER.[284] The faulty utilization of rapeseed oil in the rat may well be related to its composition. It contains 40 to 50% trierucin.[285] Since no failure in lipolysis was noted by DEUEL *et al.*,[281] the low digestibility may be ascribed to the difficulty in absorption of erucic acid.

The poor digestibility of rapeseed oil in the rat is in striking contrast to its behaviour in man. HOLMES[261] originally reported a figure of 99 for the coefficient of digestibility in man. Although this investigator did not take into consideration the possible excretion of split products as soaps, the failure to do so cannot be the cause of the divergency between the results for the rat and for

Table 18. Average coefficients of digestibility of some fats in several species of animals

Fat fed	Melting point	Coefficients of digestibility				
		Guinea pig[273]	Rat	Rabbit	Sheep	Dog
	°C					
Beef tallow	—	72·0	—	—	—	—
Butter	34·5	91·0	88·3[274]*	—	—	—
Butter	34·5	—	90·7[274]†	—	—	—
Castor oil	—	90·2	98·0[275]	92·1[273]	99[273]	—
Cacao butter	28	—	63·3[274]*	—	—	—
Cacao butter	28	—	81·6[274]†	—	—	—
Coconut oil	26	94·0	98·9[274]*	—	—	—
Coconut oil	26	—	96·5[274]†	—	—	—
Coconut oil	25	—	97·5[116]	—	—	—
Coconut oil, hydrogenated	35	—	94·5[116]	—	—	—
Cod-liver oil	—	93·8	—	—	—	—
Corn oil	—	86·5	97·5[274]*	—	—	99[276]
Corn oil	—	—	98·3[274]†	—	—	—
Cottonseed oil	—	87·4	94·8[277]	91·2[273]	94[273]	99[276]
Cottonseed oil, hydrogenated	38	—	94·4[116]‡	—	—	—
Cottonseed oil, hydrogenated	46	—	83·8[278]	—	—	—
Cottonseed oil, hydrogenated	54	—	68·7[278]	—	—	—
Cottonseed oil, hydrogenated	62	—	30·2[116]§	—	—	—
Cottonseed oil, hydrogenated	65	—	24·0[278]	—	—	—
Crisco	43	73·8	97·3[279]	91·0[273]	94[273]	—
Lard	37	75·2	96·6[279]	—	—	—
Lard	34	—	94·5[116]	—	—	—
Lard, bland	48	—	94·3[279]	—	—	—
Lard, hydrogenated	55	—	63·2[279]	—	—	—
Lard, hydrogenated	61	—	21·0[279]	—	—	—
Margarine fat	34	—	97·0[279]	—	—	99·7[280]
Margarine fat	—	—	97·0[281]	—	—	—
Mutton tallow	47	79·8	74·6[274]*	—	—	—
Mutton tallow	47	—	84·8[274]†	—	—	—
Neats foot oil	—	93·5	—	—	—	—
Oleo stock	48	—	74·0[274]*	—	—	—
Oleo stock	48	—	86·7[274]†	—	—	—
Olive oil	—	94·5	98·4[116]	—	—	—
Peanut oil, crude	—	—	97·6[277]	—	—	—
Peanut oil, refined	—	91·8	96·4[277]	—	—	—
Peanut oil, hydrogenated (blended)	39	—	92·6[277]	—	—	—
Peanut oil, hydrogenated (blended)	39	—	91·4[277]	—	—	—
Perilla oil	—	—	97·0[116]	—	—	—
Perilla oil, hydrogenated	67·5	—	6·0[116]	—	—	—
Rapeseed oil	—	—	82·0[281]	—	—	—
Salmon oil	—	94·0	—	—	—	—
Sardine oil	—	—	98·3[282]	—	—	—
Soybean oil	—	94·5	98·5[274]*	—	—	—
Soybean oil	—	—	98·3[274]†	—	—	—

* Fat fed at 5% level.
† Fat fed at 15% level.
‡ Average of 9 experiments with fat fed at 10%, 25%, and 55% of diet.
§ Average of 6 experiments with fat fed at 10% and 25% of diet.

man. DEUEL and collaborators,[257] who included the determination of faecal soaps in their studies, obtained a coefficient of digestibility in man identical with that reported by HOLMES. One can only conclude that, in the case of rapeseed oil, a variation obtains between the rat and man. At present, there is no adequate explanation for this difference.

The results on the guinea pig consistently present a third variation in the utilization of fat by lower animals as compared with man. Although cod-liver, olive, and soybean oils are digested to about the same extent in the case of the guinea pig as in other animals (including man), the coefficient of digestibility of corn, cottonseed, and peanut oils appears to be somewhat depressed in this ruminant.[272] Lard has been found to be digested to the extent of only 75·2% in the guinea pig, as contrasted with 96·6% in the rat[279] and 97·0% in man.[265]

5. *The digestibility of simple triglycerides and fatty acids*

A number of pertinent studies have been made on the digestibility of simple triglycerides and fatty acids in several species. The results of these studies are tabulated in Table 19.

Table 19. Average coefficients of digestibility of simple triglycerides and fatty acids in rats, dogs, and guinea pigs

Triglyceride or fatty acid fed	*Melting point*		*Coefficient of digestibility*		
	Pure substance	*Mixture fed*	*Rat*	*Dog*	*Guinea pig*
	°C	°C			
Trilaurin	49	—	97·3[119]	—	—
Trimyristin	56	—	76·6[119]	—	—
Tripalmitin	66·5	—	27·9[119]	95[284]	—
Tripalmitin	—	45	84·0[118]*	—	—
Tripalmitin	—	51	82·0[118]†	—	—
Tristearin	70	—	18·9[119]	10[287]	—
Tristearin	—	55	6·0[118]*	—	—
Tristearin	—	59	8·0[118]†	—	—
Lauric acid	44	32	81·5[118]‡	—	—
Myristic acid	53	44	81·9[118]‡	—	—
Palmitic acid	63	—	35·6[119]	82[284]	—
Palmitic acid	—	37	39·6[118]*	—	—
Palmitic acid	—	44	37·1[118]†	—	—
Palmitic acid	—	48	31·2[118]§	—	—
Palmitic acid	—	53	23·8[118]‡	—	—
Stearic acid	69	—	15·8[119]	—	—
Stearic acid	—	43	9·4[118]*	—	—
Stearic acid	—	51	13·3[118]†	—	—
Stearic acid	—	55	21·0[118]§	—	—
Stearic acid	—	59	19·6[218]‡	—	—
Oleic acid	—	—	95·4[273]	—	95·0[273]
Elaidic acid	—	—	95·6[273]	—	55·6[273]

* The fat mixture consisted of 5% simple triglyceride or fatty acid and 95% olive oil.
† The fat mixture consisted of 10% simple triglyceride or fatty acid and 90% olive oil.
‡ The fat mixture consisted of 25% of fatty acid and 75% of olive oil.
§ The fat mixture consisted of 15% of fatty acid and 85% of olive oil.

In the absence of other fats in the diet, trilaurin was found to be completely utilized, while trimyristin, tripalmitin, and tristearin were progressively less effectively utilized in the rat.[119] In the experiments of HOAGLAND and SNIDER,[118] in which the triglycerides were fed as 5 or 10% solutions in olive oil, trilaurin and trimyristin were practically completely utilized, while the coefficient of digestibility of tripalmitin was calculated as 84. On the other hand, the digestibility of tristearin was estimated to be only 6 and 8%, respectively, when fed as a 5% or 10% solution in olive oil. Although the results of LYMAN[286] indicate a high digestibility for tripalmitin in the dog, ARNSCHINK[287] reported a minimum utilization of tristearin in this species.

In most cases, fatty acids are poorly utilized when administered as such or in solution in olive oil. Palmitic acid was shown to have a relatively high digestibility in the dog; this is in line with the high utilization of tripalmitin in that species. However, this result is in sharp contrast to the results on rats.

PAUL and MCCAY[272] have demonstrated an interesting species difference in the utilization of oleic acid and its *trans*-isomer, elaidic acid. Whereas the rat can utilize both of these acids equally well, the guinea pig was able to absorb elaidic acid only to the extent of 55·6%, in contrast to the complete digestibility of oleic acid in this species (95%).

6. *Factors influencing the digestibility of fats*

a. The effect of age and sex

No systematic studies have been made on the effect of age or sex on the digestibility of fats. However, it is evident from the studies of HOLT and his coworkers[263], [288], [289] that the digestibility of this foodstuff is less satisfactory in infants and young children than it is in older children and adults. GORDON and MCNAMARA[290] noted a high excretion of fat in the faeces of premature infants, while WILLIAMS[291] and HARRISON and SHELDON[292] have reported that even somewhat older children have a slightly lower digestibility coefficient for fats than do adults.

b. The effect of species

Considerable differences in the digestibility of fats with high melting points, of castor oil, of rapeseed oil, and of lard have been noted between the levels for man, rats, guinea pigs, and for other animals, respectively. These are listed in Tables 16 to 19.

c. The effect of melting point

It has generally been reported that, for fats melting above some critical temperature, which is approximately 50°C, an inverse relationship obtains between the melting point of the fat and the coefficient of digestibility. CHENG and her associates[119] have reported that this relationship exists in the case of the simple triglycerides (Table 19). Fig. 5 illustrates the relationship between the percentage of unabsorbed fat and the melting point of the fat.

In analyzing the relationship of the melting point of a fat to its digestibility in

rats, DEUEL[293] has shown that, in the case of the higher melting fats, the greatest increase in the excretion of fat occurs in the soap fraction. These results are summarized in Table 20.

PAU and MCCAY[273] state that the melting point of fat is a determining factor, insofar as utilization is concerned, in the guinea pig but not in the case

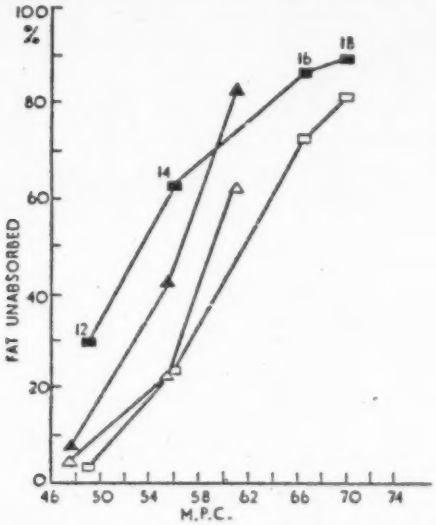

Fig. 5. The relationship of melting point to digestibility of simple triglycerides (solid square), and of samples of hydrogenated lard (solid triangle), when calcium and magnesium were present in the diet. The result of the tests with calcium-magnesium-low diets is represented in each case by similar characters which are not filled in. The numbers beside each character indicate the number of carbon atoms in the fatty acid component of the simple triglycerides.[118]

Table 20. Comparison of the effect of melting point on the digestibility of some natural and hydrogenated fats in rats, and the distribution of excreted fats between neutral fats and soaps[293]

Fat fed	*Melting point*	*Fat intake*	*Faecal fat*		*Coefficient of digestibility**
			Neutral fat + fatty acids	*Soaps*	
	°C	*gm*	*gm*	*gm*	
Fat-free diet . . .	—	0	0·18	0·088	—
Margarine fat . . .	34	10·7	0·24	0·30	97·0 ± 0·4
Prime steam lard . .	37	12·2	0·21	0·48	96·6 ± 1·4
Crisco	43	12·3	0·23	0·38	97·3 ± 0·3
Bland lard . . .	48	12·8	0·34	0·68	94·3 ± 1·8
Hydrogenated cottonseed oil .	46	14·9	0·73	2·09	83·4 ± 1·4
	54	12·3	0·62	3·80	68·7 ± 2·7
	65	15·7	6·31	6·93	24·0 ± 2·6
Hydrogenated lard . .	55	13·5	0·58	4·90	63·2 ± 1·2
	61	13·2	2·21	8·89	21·0 ± 2·6

* Including the Standard Error of the Mean.

of rabbits and sheep. On the other hand, HOAGLAND and SNIDER[274] have pointed out that their experiments do not support the thesis that a definite relationship exists between melting point and digestibility. Thus, it was noted that mutton tallow (m.p., 47°C) had a higher digestibility than cacao butter (m.p., 28°C). Moreover, cacao butter, butterfat, and coconut oil, each melting below body temperature, had widely different coefficients of digestibility.[274] HOAGLAND and SNIDER[118] are of the opinion that the stearic acid content rather than the melting point is the factor which controls digestibility. However, the experiments of MATTIL and HIGGINS,[294] summarized in Table 21, speak against this hypothesis. One must conclude that, whereas melting point is an important consideration in determining the digestibility of a fat, it is not the sole factor, and other conditions such as composition must influence it.

d. The effect of the structure of the fat

Natural fats are known to exist largely in the form of mixed triglycerides rather than as simple triglycerides. The question is pertinent as to whether the digestibility of a fat depends upon its constituent acids or whether it is a function of the arrangement of such acids in the triglyceride molecules. The experiments of MATTIL and HIGGINS[294] have definitely proved that the second possibility is the one which is of importance in establishing the utilization of the fat. Thus, as shown in Table 21, the extent of digestibility varies markedly, depending

Table 21. Digestibility of oleic and stearic acids when fed as simple or as mixed triglycerides[294]

Fat fed	*Fat*		*Coefficient of digestibility*
	In food	*In faeces*	
	gm	gm	
Tristearin, 10%; triolein, 5%	51·0	29·5	42·2
	79·2	48·4	37·6 } Av. 39·9
Distearomonoolein, 15%	94·2	39·7	57·9
	24·5	9·0	63·3 } Av. 60·6
Tristearin, 5%; triolein, 10%	87·5	27·4	68·7
	35·9	11·3	68·5 } Av. 68·6
Monostearodiolein, 15%	89·4	24·3	72·8
	14·6	3·9	73·3 } Av. 73·0

upon whether the fatty acids are given to the animal in the form of mixtures of the simple triglycerides or as mixed triglycerides containing a similar proportion of fatty acids.

e. The effect of polymerization of the fat

When fats are heated at 275° to 300°C over a period of time, polymerization occurs. As a result of this treatment, the odour and taste characteristic of

unsaturated glycerides are removed; at the same time, the oils develop a considerable degree of viscosity. This alteration in properties is associated with a change in structure, as is indicated by the fact that the degree of unsaturation, as determined from the iodine number, decreases to a considerable degree as polymerization progresses.

Several studies have recently been published which indicate that polymerization may influence the physiological as well as the physical properties of a fat. LASSEN, BACON, and DUNN[282] reported that the coefficient of digestibility of sardine oil, heated at 250°C for various periods of time, was depressed from 98·3 for the untreated oil (iodine value 177·7), the values for the several fractions being as follows: iodine value, 155·5, 96·0; iodine value, 138·1, 89·5; and iodine value, 124·1, 84·8. ROY[295] reported similar but less pronounced effects on samples of cow ghee, buffalo ghee, lard, and hydrogenated peanut oil when the fats were subjected to 250°, 275°, or 300°C. No effect was noted when a temperature of 200°C was employed, and the values were only slightly altered at 250°C.

f. The effect of foodstuffs fed concomitantly with fat

There are a number of ways in which the digestibility of fat may be influenced by other foodstuffs present at the time of digestion and absorption. The most important substances in this category are inorganic salts, which form insoluble soaps and thus prevent absorption of a considerable proportion of the fatty acids. Mineral oil has already been mentioned as an indigestible substance which may reduce absorption by removing some absorbable lipids in solution via the intestine. Moreover, when gastric or intestinal irritants are present in the foods, the length of stay of the food in the intestine may be so curtailed as to preclude a normal utilization.

(*a*) *The effect of calcium salts in the diet*—Although CHENG and her associates[119] reported that no appreciable effect was exerted on the digestibility of bland lard when calcium salts were present in the diet, the extent of digestibility of most fats was decreased when these salts were included in the ration. These data are summarized in Table 22.

The presence of calcium has a more pronounced effect on the digestibility of trimyristin and trilaurin than on that of the other fats studied. It was also reported by CHENG and associates[119] that the extent of digestibility of trilaurin was proportional to the quantity of calcium in the diet. Thus, the following coefficients of digestibility were reported in diets containing 6·1, 2·5, 1·17, and 0 mg of calcium per gram of food, respectively: 70·5, 87·2, 89·5, and 97·3.

GIVENS[296] was among the first to report that calcium absorption was decreased when the fat utilization was poor. The extent of the soap formation was believed by BOSWORTH and co-workers[297] to be dependent upon the proportion of ionized calcium, although the amount of soap lost in the faeces was obviously a function of the solubility of the calcium soap. Since calcium oleate is more readily soluble in bile than is calcium palmitate or calcium stearate, it will disappear in a larger proportion than the last two soaps,

Table 22. The effect of inclusion of calcium salts in the diet on digestibility of fats[119]

Fat fed	Melting point	Ca-free diets				Diets containing Ca			
		Fat ingested	Neutral fat + fatty acid in stool	Soaps in stool	Coefficient of digestibility	Fat ingested	Neutral fat + fatty acid in stool	Soaps in stool	Coefficient of digestibility
	°C	gm	gm	gm		gm	gm	gm	
Bland lard	47·8	12·2	0·39	0·54	95·8	13·3	0·25	1·16	92·4
Blended lard*	47·9	12·5	0·37	0·57	95·6	14·1	0·24	1·43	91·7
Blended lard†	55·2	9·1	1·33	1·16	80·0	10·7	1·06	2·97	66·2
Hydrogenated lard‡	55·4	9·2	1·17	1·40	77·9	11·8	0·51	4·67	58·0
Hydrogenated lard‡	61·0	10·6	5·98	1·99	38·0	11·0	3·78	6·03	17·3
Trilaurin	49	8·8	0·16	0·29	97·3	8·9	0·25	2·72	70·5
Trimyristin	56	9·7	1·49	1·33	76·6	12·8	1·12	7·46	37·7
Tripalmitin	66·5	9·8	7·51	1·27	27·9	9·1	6·40	2·27	12·8
Tristearin	70	9·0	7·97	0·88	18·9	9·5	7·50	1·90	10·6
Palmitic acid	63	9·1	4·98	2·29	35·6	9·7	3·89	4·60	19·8
Stearic acid	69	9·6	6·92	2·78	15·8	9·8	3·99	5·53	14·4
Monostearin	59·9	9·5	4·00	1·84	47·4	10·1	3·22	5·55	20·7

* One part hydrogenated lard to 9 parts prime steam lard. Reported in unpublished communication.
† One part hydrogenated lard to 2 parts prime steam lard.
‡ Whole sample partially hydrogenated.

even in the presence of an excess of calcium. Similar data are recorded by BOYD *et al.*[298]

The depressing effect of a high-calcium intake on the utilization of fats occurs primarily in the case of fats having a relatively high stearic acid content. According to the extensive experiments carried out by the U.S. Department of Agriculture on the digestibility of fat by human subjects, practically all fats and oils of animal and vegetable origin with melting points under 50°C were utilized to the extent of 93% or better, in spite of the fact that the blancmange used in the diet was made from skim milk, which has a relatively high calcium content In most of the tests recorded in Table 18 on animals other than man, calcium was likewise present in the diet. It is only in those natural fats and hydrogenated fats in which a sufficient stearic acid content is present to increase the melting point of the fat above 50°C that the deleterious effect of high calcium diets becomes evident.

(*b*) *The effect of protein*—Protein is a second dietary component which may modify the digestibility of fat. BARNES, PRIMROSE, and BURR[299] were the first to call attention to the fact that fats have a lower coefficient of digestibility in rats on a low-protein diet (14%) than on a dietary regimen with a higher protein content (30%). These results have been confirmed by SAVAGE[300] and by SEVERANCE.[277] On the other hand, several other investigators[301–303] have failed to demonstrate any alteration in the digestibility of fats in dogs or in human subjects. Table 23 records some of the positive data.

Table 23. The comparative digestibility of several fats in the case of rats receiving low- or high-protein diets

Fat fed	Sex	Low-protein diet			High-protein diet		
		Fat intake	Faecal fat	Coefficient of digestibility	Fat intake	Faecal fat	Coefficient of digestibility
		gm	%		gm	%	
Steam lard[299]	M	2·09	6·52	97·6	2·39	4·06	98·4
	M	1·59	16·74	91·9	2·07	10·20	95·4
	F	1·77	5·28	98·0	1·86	1·51	99·5
	F	1·82	19·11	94·0	1·36	7·88	96·7
Standard butter[299]	M	1·80	20·26	89·3	1·89	9·41	95·9
	F	1·63	18·86	92·1	1·61	6·09	97·7
Special butter spread[299]	M	2·38	17·61	92·4	2·33	10·41	96·2
	F	2·00	19·47	92·4	1·86	10·67	96·0
Peanut oils:[277]							
Crude	M	2·63	25·48	95·5	1·96	11·89	97·6
Refined	M	2·64	25·98	95·5	2·38	14·35	97·3
Hydrogenated	M	2·80	47·10	86·4	2·19	36·70	89·4
Blended hydrogenated	M	2·70	38·40	90·8	2·34	29·10	91·7

g. The effect of emulsifying agents

Although it is impossible to improve the digestibility of highly utilized fats by the use of emulsifying agents, AUGUR and co-workers[278] were able to demonstrate a marked elevation in the coefficients of digestibility of high-melting hydrogenated cottonseed oil samples in the presence of lecithin. The coefficients of digestibility of these samples in rats without and with lecithin were as follows: sample melting at 46°C, 83·8 and 87·9; sample melting at 54°C, 68·7 and 82·8; and the sample melting at 65°C, 24·0 and 44·2.

"Tween 80" (PSM or polyoxyethylene sorbitan monooleate), which is an especially effective emulsifying agent, was shown to increase fat utilization in patients whose capacity for fat utilization was low.[304]

3. Absorption Rate of Fats as Related to their Nutritional Value

Although absorption and digestibility are considered by many people to be identical, they actually represent different physiological functions. Absorption is measured in terms of rate, while digestibility records the proportion of ingested material which is utilized. Ordinarily, fats which are quickly absorbed have a high coefficient of digestibility. On the other hand, the converse may not necessarily be true.

Although no differences may be observed between the quantities of two fats which can be utilized, as determined from digestibility studies when the fats are taken in moderate amounts, an entirely different picture may be obtained if they are fed in large doses. Thus, the maximum amount of a fat which may be

taken without causing a digestive disturbance varies widely. If the fat is one which is absorbed slowly, diarrhoea may occur on the administration of relatively small quantities; in the case of a fat having a more rapid rate of absorption, tolerance is greater before the onset of the diarrhoea. It is therefore evident that absorption rates and digestibility coefficients, although related, afford information on independent physiological responses to fat.

1. *Methods for expressing the rate of absorption*

There are several possible methods by which the rate of absorption may be expressed. One of these procedures, employed by STEENBOCK, IRWIN, and WEBER,[305] simply involves recording the percentage of the original dose absorbed during a 4-hr period. As long as the size of the animals, the time of absorption, and the dosage employed are uniform, one would expect to obtain fairly consistent results by this procedure. However, this index loses much of its value if any one of the above conditions is varied.

A second procedure for comparing the rates of absorption is to base them upon the quantity per unit surface area of body surface per hour.[283] In testing this method for evaluating absorption, it was demonstrated that fairly constant rates were obtained for absorption of fats in rats varying widely in size, and when different dosages were employed over several time intervals. On the other hand, no consistent results could be obtained by the use of the index proposed by STEENBOCK *et al.*[305] under the above conditions. Surface area is thus a biometric measurement important in assessing not only basal metabolism and the absorption of glucose, but also fat absorption.

2. *Comparative absorption rates of different fats*

The results of absorption of 1·5 ml doses of some common fats by adult rats approximately uniform in size, as calculated by the index of STEENBOCK *et al.*,[305] are indicated in Table 24, while some comparable studies in which

Table 24. The mean percentages of fats absorbed by previously fasted male rats at several periods after feeding 1·5 ml of the various fats by stomach tube[305]

Fat fed	*Absorption* in % of original dose at:*				
	2 hours	*4 hours*	*6 hours*	*8 hours*	*12 hours*
Butterfat	36·2 ± 1·6	60·3 ± 1·2	77·2 ± 2·0	91·2 ± 1·1	97·4 ± 0·4
Butter oil	37·4 ± 2·3	71·0 ± 1·2	86·4 ± 1·7	95·6 ± 1·0	—
Cod liver oil	40·8 ± 1·4	67·7 ± 1·9	79·7 ± 1·5	89·2 ± 0·7	98·2 ± 0·4
Corn oil	28·9 ± 0·8	58·3 ± 0·9	71·4 ± 2·1	94·4 ± 0·7	97·9 ± 0·3
Halibut liver oil	39·4 ± 1·6	70·2 ± 2·0	78·1 ± 1·3	85·4 ± 0·9	—
Lard	24·1 ± 0·8	57·0 ± 1·5	67·5 ± 1·5	92·3 ± 0·9	97·8 ± 0·4
Shortening A	26·6 ± 1·5	53·8 ± 1·6	68·5 ± 1·7	86·0 ± 1·3	98·6 ± 0·3
Shortening B	27·1 ± 1·8	52·8 ± 2·4	71·1 ± 1·5	85·6 ± 1·2	99·6 ± 0·1

* Including Probable Error of the Mean.

Table 25. The absorption of natural fats and hydrogenated fats when fed to rats at a level of 300 mg per 100 cm² of body surface

Fat fed	Sex	Melting point	Absorption in mg/100 cm²/hr*		
			3 hours	6 hours	8 hours
		°C			
Butterfat[283]	M	—	49·6 ± 2·7	42·6 ± 2·1	—
	F	—	52·6 ± 5·1	41·6 ± 2·1	—
Coconut fat[283]	M	—	42·0 ± 4·5	43·2 ± 2·0	—
Corn oil[306]	F	—	—	—	44·9 ± 1·6
Cottonseed oil[278], [283]	M	—	39·8 ± 2·9	39·7 ± 1·5	—
	F	—	38·5 ± 0·0	43·3 ± 1·3	—
Prime steam lard[279]	F	—	35·0 ± 1·9	38·3 ± 2·0	—
Rapeseed oil[283]	M	—	38·5 ± 3·0	30·0 ± 1·8	—
	F	—	37·0 ± 1·5	26·2 ± 1·9	—
Crisco[279]	F	43	37·1 ± 2·0	34·3 ± 0·6	—
Hydrogenated cottonseed oil[278]	F	46	26·5 ± 1·8	24·7 ± 2·0	—
	F	54	18·0 ± 1·8	8·5 ± 0·9	—
Hydrogenated lard[279]	F	48†	34·5 ± 1·7	31·4 ± 1·6	—
	F	55	20·7 ± 3·5	21·6 ± 3·5	—
Margarine fat[233]	M	34	44·5 ± 3·3	40·1 ± 1·9	—
Margarine fat[280]	M	34	41·7 ± 0·0	36·5 ± 0·0	—
Margarine fat[283], [307]	F	34	36·3 ± 1·0	39·7 ± 1·8	—
Margarine fat[280], [306]	F	34	43·2 ± 0·0	36·0 ± 0·0	42·8 ± 1·4

* Including the Standard Error of the Mean.
† Bland lard. This is a mixture of unhydrogenated lard and lard hydrogenated to a melting point above 48°C.

absorption is based upon milligrams per 100 cm² per hour are given in Table 25.

The rates of absorption of butterfat, coconut, corn, and cottonseed oils and prime steam lard, as well as that of margarine fat (Table 25), are in the same range in the 6- and 8-hr tests, although, in the studies at the 3-hr interval, butter appears to be absorbed slightly more readily. On the other hand, rapeseed oil is absorbed more slowly than are other oils; this discrepancy becomes particularly evident in the tests carried out over longer periods. This is in line with the observation that rapeseed oil is poorly utilized in the rat.[119]

In the case of the hydrogenated fats other than a margarine fat, Crisco, a hydrogenated cottonseed oil melting at 46°C, and a hydrogenated lard melting at 48°C are absorbed at a slightly lower rate. On the other hand, the absorption rates of a hydrogenated lard melting at 55°C and of a hydrogenated cottonseed oil melting at 54°C are definitely lower than those of other fats. In spite of the wide discrepancies in absorption rates, most of the fats are ultimately equally well utilized, and only small amounts are lost in the faeces. Exceptions to this complete utilization include rapeseed oil (mentioned above), the high-melting hydrogenated cottonseed oil,[278] and hydrogenated lard.[279]

In most cases, the rate of absorption is highest for the shorter periods, and decreases toward the end of the test period. There are several possible explanations for this phenomenon. In the first place, the highest concentration of fat

obtains during the early period, and this presumably increases the rate of absorption to some extent.[283] Moreover, the rate of absorption of the several glycerides, in any single natural fat, differs according to their respective fatty acid content. Presumably this would mean that the most rapidly absorbed glycerides would be utilized first. This explanation might account for the faster absorption of butter during the earlier periods when the short-chain glycerides were first being utilized. Also, this same reasoning would apply to the results with rapeseed oil. During the early period, when the C_{18}-triglycerides are being absorbed, the rate of absorption of rapeseed oil compares favourably with that of other natural fats. However, when the residue becomes largely trierucin, the speed of absorption is markedly retarded.

It is a moot question as to what relationship exists between the rate of absorption and the nutritional value of a fat. In the first place, when fats are absorbed at a rapid rate, large amounts can be tolerated without producing a diarrhoea or, presumably, other unfavourable symptoms. On the other hand, such rapid absorption might tend to bring about a greater alimentary lipemia than would be observed in the case of more slowly absorbed fats. Moreover, the advantages of the greater satiety which appears to be associated with a longer retention of a foodstuff like fat in the gastrointestinal tract, as compared with carbohydrate or protein, are counteracted by the shortening of the absorption period.

In the case of fats which are more slowly absorbed, one can argue that the presence of fat in the gastrointestinal tract over a longer period has a beneficial effect, in that it extends the period of absorption, and thus may entail less of a burden on the organism during any one period. It is only when the rate of absorption of a fat is so slow that the process cannot be completed while the fat is in the part of the gut where the absorption is actively taking place (and hence some is lost in the faeces) that a slow rate of absorption becomes a factor in lowering the nutritional value of a fat. Annegers and Ivy[308] reported that an increase in the proportion of fat prolongs the gastric evacuation time. No significant differences were found between the effect of lard and of vegetable oil, respectively, on gastric emptying time.

3. *Factors affecting the rate of absorption of fats*

a. The effect of age

According to Irwin *et al.*,[309] neither age nor sex is a factor in regulating the rate of absorption of fats, at least within the range of their experiments, namely from 4 to 7 months. However, with the technique employed by these investigators, which involves feeding 1·5 ml of the fat, irrespective of the size of the rat, the index of absorption would most certainly have been much lower had very young rats been tested. Thus, Deuel and collaborators[283] reported that, when 1100 mg of fat were fed to male rats weighing 270 gm, 43·2% was absorbed in 3 hr, compared with a value of 23·1% when the same dosage was administered to young rats weighing an average of 63 gm. However, when the absorption index was based upon surface area, the values for these groups become 43·3 and

56·1 mg per 100 cm² of surface area per hour, respectively. Moreover, when a standard dosage of 300 mg per 100 cm² of body surface was used, the comparative values for males were 43·3 ± 1·8 mg (270 gm rats) and 53·8 ± 2·6 mg (74 gm rats); the results obtained with females were 38·1 ± 3·2 mg (150 gm rats) and 43·7 ± 2·1 mg (64 gm rats). One can only conclude that the rate of absorption of fats is not influenced by the size of the rat (and hence the age) over a fairly wide range if the results are expressed in terms of surface area. The size of the dosage would appear to be of less consequence, although the most consistent results were obtained when this factor, also, was adjusted to surface area.

In the case of man, however, extreme variations in age have been shown to influence the rate of absorption of fat. SOBEL and co-workers,[310] as well as TIDWELL *et al.*[311] have demonstrated that newborn babies and infants under one year of age absorb fats quite inefficiently, as compared with older children. On the other hand, BECKER and associates[312] reported that fat is absorbed or metabolized more slowly in the aged than in younger subjects. Thus, the chylomicron count was found to remain elevated for a prolonged interval after the feeding of fats to aged subjects.

b. The effect of sex

In spite of the fact that sex plays a role in the metabolism of fats, as well as in the requirement for essential fatty acids (p. 104), it does not, apparently, exert any effect on the rate of absorption of fats.[283], [309] IRWIN *et al.*[309] have likewise reported that pregnancy does not influence the rate of absorption of fats.

c. The nature of the fat

There is no doubt that the nature of the fat is of prime importance in establishing the rate of absorption. If one subscribes to the Frazer hypothesis of fat absorption, then variations in physical properties may well be the factors responsible for differences in absorption rate. On the other hand, if fats must be split prior to absorption, according to the Verzár theory, then differences in absorption rate between different fats may reflect variations in the rate of hydrolysis, as well as in the rate of absorption, of the resultant fatty acids.

(*a*) *The rate of absorption of simple triglycerides*—Although most of the triglycerides found in naturally-occurring fats are mixed, information on the effect of the type of fatty acid chain on absorption can best be obtained with simple triglycerides. These data can be obtained only for triglycerides with fatty acids of C_{12} or lower, since the high melting point of the longer-chained compounds practically precludes their administration. It is well known that such high-melting fats as tripalmitin and tristearin are utilized only to a minor extent. Thus the rate of absorption would be minimal. Table 26 gives a summary of absorption tests on simple triglycerides composed of C_2 to C_{12} acids.

There are two interesting features to be noted in Table 26. The first one is the extremely rapid absorption rate of triacetin and tributyrin. It is also evident that, as the number of carbons in the even-chain acids increases, there is a progressive decrease in the rate of absorption. The second phenomenon of note

Table 26. The absorption rates of simple triglycerides in fasting rats over three-hour periods[313]

Fat fed	*Number of carbons*	*Absorption in mg/100 cm²/hr**	
		Even-chain fats	*Odd-chain fats*
Triacetin	2	68·1 ± 1·4 (12)	—
Tripropionin	3	—	31·4 ± 2·1 (12)
Tributyrin	4	65·0 ± 2·5 (10)	—
Triisovalerin	5	45·7 ± 2·5 (6)†	—
Trivalerin	5	—	32·9 ± 2·3 (9)
Tricaproin	6	54·5 ± 1·5 (9)	—
Triheptylin	7	—	28·0 ± 1·6 (10)
Tricaprylin	8	45·9 ± 4·1 (8)	—
Tricaprin	10	‡	—
Trilaurin	12	21·9 (5)	—

* Including Standard Error of the Mean. The figures in parentheses represent the number of tests.
† Forked-chain acid classed as even-chain.
‡ Diarrhoea in all 20 tests.

is the marked retardation in absorption rates of the triglycerides containing an odd number of carbons as compared with the triglycerides having an even number of carbons. However, tripropionin (C_3), trivalerin (C_5), and triheptylin (C_7) all have practically the same absorption rates; they do not exhibit the progressive decrease found for tributyrin (C_4), tricaproin (C_6), and tricaprylin (C_8). Triisovalerin, although containing an odd number of carbons, appears to fall in line with the even-chain acids rather than with the odd-chain acids.

(*b*) *The rate of absorption of fatty acids*—It is not known whether the behaviour of the simple triglycerides, as regards absorption, is due to some property inherent in the triglyceride molecule, or whether it is related to the fatty acids themselves. The results in Table 27 indicate that the soaps exhibit, to some extent, the same pattern of absorption as do the corresponding triglycerides. Although this would at first seem to support the Verzár Lipolytic Theory concerning the mechanism of the absorption of fats, it should be recalled that the Partition Theory of Frazer likewise predicates the hydrolysis of the triglycerides which contain water-soluble fatty acids. Moreover, it is not certain that the administration of the lower fatty acids in the form of soaps involves a strictly physiological situation.

The results in general support the thesis that variations in absorption rate of the simple triglycerides are related to the speed of absorption of the fatty acids. However, acetate exhibits a slow rate of absorption, in contradistinction to the rapid rate of absorption of triacetin. This alteration may, in part, be due to the use of the sodium salts of the shorter-chain fatty acids, since the free acids are too corrosive to be employed. The lower rate of removal of the odd-chain acids, propionic, valeric, and heptylic, as compared with the corresponding even-chain values, can be observed in the case of the soaps. The short duration of the tests when the soap solutions were employed was due to the fact that it was impossible

Table 27. The rate of absorption of the lower fatty acids by female rats[314]

Fatty acid fed	*Absorption in mg/100 cm²/hr**	
	1 hour	*3 hours*
Acids fed as sodium salts in 20% aqueous solutions in doses of 100 mg/100 cm²		
Acetic	26·6 ± 1·0 (10)	—
Propionic	21·4 ± 1·2 (16)	—
Butyric	39·7 ± 1·5 (18)	—
Butyric[306]	45·0 ± 2·6 (16)	—
Valeric	23·3 ± 1·9 (11)	—
Caproic	38·0 ± 1·7 (11)	—
Heptoic	25·8 ± 1·5 (20)	—
Acids fed in free state in doses of approximately 100 mg/100 cm² (in 1-hour tests) and 200 mg/100 cm² (in 3-hour tests)		
Caprylic	37·3 ± 1·5 (23)	46·0 ± 1·7 (10)
Nonylic	34·4 ± 2·3 (24)	32·8 ± 1·3 (23)
Capric	19·2 ± 1·5 (22)	22·6 ± 2·1 (11)
Undecylic	21·3 ± 3·4 (15)	21·4 ± 0·5 (9)
Lauric	2·7 ± 1·2 (10)	3·8 ± 0·7 (10)
Tridecylic	20·8 ± 3·2 (15)	†

* Including Standard Error of the Mean. Figures in parentheses represent the number of tests included in the average.
† Diarrhoea developed in 16 of 18 rats before 3 hours.

to administer doses of sufficient magnitude to insure a residue in the gut after a period longer than one hour.

(c) *The effect of the melting point of the fat on the rate of absorption*—Probably the most important factor which influences the rate of absorption is the physical state of the fat which, in turn, is related to its melting point. Few observations are available on the rate of absorption of fat melting at over 50°C, because of the difficulty of administering the fat in liquid form without killing the test animal. However, in the tests on digestibility in which the fat was eaten as part of the diet mixture, the utilization may in some cases be so low as to indicate a rate of absorption approaching 0. Intermediate values have been noted for hydrogenated lard (melting at 55°C) and for hydrogenated cottonseed oil (melting at 54°C) (see Table 25).

d. The role of the adrenocortical hormones

It is now recognized that the hormones of the suprarenal cortex are important in fat absorption. Verzár and Laszt[315], [316] were the first to demonstrate the influence of the adrenal glands on fat absorption. In an extensive series of investigations, these workers showed that the absorption of fat in rats was inhibited by adrenalectomy, and that the normal function of the gland, in

respect to fat absorption, could be restored by the administration of cortical extract, but not of ascorbic acid, which is one of the chief active agents of the adrenal medulla. It was postulated that the adrenocortical hormones control the phosphorylation of fat in the intestinal mucosa; this reaction was considered to be essential for the absorption of fat. The mechanism was likewise related to the presence of riboflavin (vitamin B_2), since the activity of the adrenal cortex was found to be diminished in riboflavin deficiency.[317]

On the other hand, BARNES and co-workers[318], [319] failed to note any decrease in the absorption of methyl esters of the fatty acids of corn oil or of corn oil itself, after the removal of the adrenal glands of rats, if the animals were maintained on salt solution. This would assign the adrenal glands to a secondary role in the absorption of fats, as had previously been demonstrated in the case of glucose. In the latter instance, if dehydration and the consequent circulatory disturbances following adrenalectomy were avoided by the administration of Rubin-Krick solution, or by that of sodium chloride solution, no disturbance in carbohydrate absorption could be noted after extirpation of the adrenal glands.[320]

However, more recent results have supported the concept of VERZÁR and LASZT that the adrenal cortex plays a primary role in fat absorption. Thus, BAVETTA and associates[307] reported that the reduction in the absorption rate of fat amounted to 38% of the normal in untreated adrenalectomized rats, while it was still 24% of the normal in salt-treated animals from which the adrenals had been removed. The lowering in absorption was associated with an accumulation of fatty acids in the intestine.[307] Both of these deficiencies were corrected by the oral administration of cortin. In later work of BARNES *et al.*[321] a decreased rate of absorption was reported for emulsified hydrogenated cottonseed oil after adrenalectomy, although no deviation from the normal was observed for certain other oils in operated rats. BAVETTA and DEUEL[306] were unable to confirm this latter work, and suggested that the failure of BARNES and co-workers[321] to note decreased absorption, in the case of a majority of the fats tested following adrenalectomy, may have been due to the fact that large (and probably old) rats were used; it has long been recognized that cortical deficiency is much more critical in younger animals than in old ones.

In spite of the fact that adrenalectomy retards the absorption of fats composed principally of C_{16} and C_{18} fatty acids, BAVETTA and DEUEL[306] reported that no difference obtained in the rate of absorption of tributyrin in normal and in salt-treated adrenalectomized rats. In later work, BAVETTA[322] found that no significant depression in absorption resulted from extirpation of the adrenal glands in the case of tricaproin (C_6) or tricaprylin (C_8). Sodium butyrate[306] and sodium caproate[322] were absorbed equally well in normal and in operated rats. However, the absorption of caprylic (C_8) and capric (C_{10}) acids, when they were fed as the free acids, was found to be decreased following adrenalectomy. It was concluded that the adrenal cortex was concerned only with the absorption of the long-chain fatty acids which are insoluble in an aqueous medium.

e. The effect of emulsifying agents

It has recently been demonstrated that the presence of emulsifying agents will increase the rate of absorption as well as the coefficient of digestibility of difficultly absorbed fats. Although some emulsifying agents will also accelerate the rate of absorption of readily absorbed fats such as limpid cottonseed oil, they ca not increase the coefficient of digestibility, in view of the fact that these fats, even without the emulsifer, are practically completely utilized. The commoner emulsifying agents in foods include lecithin, mono- and diglycerides, and polyoxyethylene sorbitan monooleate (PSM, marketed under the trade name of "Tween 80").

(*a*) *The effect of lecithin*—Commercial lecithin has been widely employed as an emulsifying agent in a variety of foods, over the past several decades. By the use of this phospholipid, it has been possible to prepare extremely fine emulsions which possess a remarkable degree of stability.

AUGUR and her co-workers[278] were able to demonstrate that fats were removed from the intestines more rapidly if they contained lecithin, than they were if this phospholipid was absent. When cottonseed oil was fed to fasting female rats, the absorption rate (expressed in milligrams per 100 cm^2 body surface per hour) was increased by lecithin from 47·8 $\pm$ 2·3 to 61·8 $\pm$ 3·7 in the 2-hr tests, and from 38·5 to 59·9 in the 3-hr experiments. The results on high-melting hydrogenated cottonseed oil were equally decisive in showing the accelerating effect of lecithin. The values for absorption of the sample melting at 46°C were as follows for the fat without and with lecithin, respectively: 3-hr tests, 26·5 $\pm$ 1·8 and 48·9 $\pm$ 3·0; 6-hr tests, 24·7 $\pm$ 2·0 and 36·7 $\pm$ 2·2. In the case of the sample of hydrogenated cottonseed oil melting at 54°C, the results were as follows: 3-hr tests, 18·0 $\pm$ 1·8 and 30·1 $\pm$ 2·8; and 6-hr tests, 8·5 $\pm$ 0·9 and 21·8 $\pm$ 1·5. It could be shown statistically that these differences were highly significant.

Another experimental procedure which has been used to demonstrate the favourable behaviour of lecithin in aiding the absorption of fat is its effect on the susceptibility to diarrhoea. When doses of fat amounting to from 300 to 600 mg per 100 cm^2 body surface were given to rats, a fairly high incidence of diarrhoea was noted. Thus, in a series of 50 tests in which cottonseed or hydrogenated cottonseed oil was used, diarrhoea occurred in 20 cases, or 40%. On the other hand, only 4 of 40 rats subjected to tests with lecithin-containing fat, fed at the same level as those described above, developed diarrhoea. This represents an incidence of 10%, or one-fourth of the control value.

(*b*) *The effect of polyoxyethylene sorbitan monooleate (PSM or "Tween* 80")—No data are available in regard to PSM comparable to that recorded above for lecithin. However, JONES *et al.*[304] reported on the fat absorption in a group of patients with nutritional difficulties secondary to subtotal gastrectomy, carried out to correct duodenal ulcer, as well as in a case of sprue. It is believed that the improvement in absorption is accomplished through the effect of PSM on surface tension, which facilitates the production of a fat emulsion composed of finer fat droplets than would otherwise be the case.

f. The effect of inhibitors

Substances such as monoiodoacetic acid and phlorhizin, which are believed to inhibit phosphorylation, have likewise been found to decrease the rate of fat or fatty acid absorption in rats.

(*a*) *Experiments with monoiodoacetic acid*—Although there is little doubt that monoiodoacetate interferes with the absorption of glucose, galactose, and fructose, in contradistinction to its inactivity in delaying the absorption of the pentoses, xylose, and arabinose,[323] there is some question as to whether or not it acts to inhibit the absorption of fat. VERZÁR and MCDOUGALL[317] reported the results on rats given 3·5 ml of olive oil without monoiodoacetic acid (A), or with doses of this compound in the amount of 0·07 to 0·1 mg per gm body weight (B). After 6 hr, the following comparative results were noted: fat absorbed, A, 1·5 gm, B, 0 gm; fat in stomach, A, 1·5 gm, B, 3 gm; fat in intestine, A, 0·5 gm, B, 0·5 gm. The failure in absorption was not due to gastric retention of fat in iodoacetate-treated rats, since these rats had an inability to absorb the fat when it was introduced directly into the intestine. Monoiodoacetic acid was found to have no inhibitory action on lipase;[324] in fact, half of the fat in the intestine of the monoiodoacetate-treated rats was found to be hydrolyzed. On the other hand, the monoiodoacetate tests have been severely criticized by KLINGHOFFER[325] as well as by ÖHNELL and HÖBER,[326] on the grounds that this substance is very toxic and produces bleeding and irreversible damage to the intestinal mucosa. It was pointed out that not only was the absorption of fat and of glucose inhibited after the administration of monoiodoacetate, but also that of the pentoses and even of sodium chloride was reduced. The absorption is not believed to be influenced by monoiodoacetate in either of the latter groups.

(*b*) *Experiments with phlorhizin*—VERZÁR and LASZT[327] were the first to demonstrate that an inhibition of fat absorption occurs in the rat following the injection of the glucoside, phlorhizin. When phlorhizin was given to rats, it was found that the absorption of fat was reduced practically to 0 over a 6-hr period, irrespective of whether the fat was given *per os* or was injected into the intestine.[328] Not only was the absorption of fat prevented but a similar inhibition in the absorption of fatty acids was noted. In the case of phlorhizin, one cannot level the same criticism as regards toxicity as applies to monoiodoacetate, inasmuch as phlorhizin is less toxic.[329]

As in the case of monoiodoacetate, the inhibitory action of phlorhizin has been ascribed to its interference with phosphorylation.[317] The difficulty resulting from both drugs is not ascribed to the inability to transfer the fatty acid to the intestinal mucosa, but rather to the absence of synthesis of the triglyceride within the cell. According to VERZÁR and MCDOUGALL, the formation of triglycerides must involve an intermediate phosphorylation which cannot occur in the presence of inhibitors. FRAZER,[330] however, discounts the validity of the experiments with phlorhizin, as well as with monoiodoacetate, as the doses used were excessively large and caused extensive damage to the intestinal mucosa.

The subject of the mechanism of fat absorption will be treated in a chapter by BERGSTRÖM in Volume 3 of this series.

4. The Comparative Nutritive Value of Vegetable and of Animal Fats

1. *General remarks*

Since fats would appear to have a primary significance, not only as sources of calories, but also for many varied physiological functions, it is of considerable importance to ascertain whether or not the need for fats can be met equally well by vegetable or by animal fats. From a chemical standpoint, vegetable and animal fats are composed of the same type of fatty acids and glycerides. However, the animal fats, in general, contain more stearic acid than do the vegetable fats, while the reverse is true insofar as the unsaturated acids, linoleic and linolenic, are concerned. There is some indication that the manner of distribution of fatty acids in the triglyceride molecules varies somewhat in the animal and vegetable fat molecules, respectively. Thus, HILDITCH[285] has pointed out that the principle of "even distribution" obtains in the case of vegetable fats, while a random arrangement is presented by the saturated acids (but not by the unsaturated acids) in the case of animal fats. However, since the animal is able to hydrolyze and to resynthesize the triglycerides, there is no reason to suppose that any variation in metabolism would obtain because of the slightly different pattern of assembling the fatty acids.

Some of the differences in nutritional value, formerly ascribed to fats, are now recognized to be related to variations in their content of fat-soluble vitamins. Whereas vitamin A as such is never found in the vegetable fats, it may, in some cases, be present in the animal fats. In the latter instance, the highest concentrations occur in the liver fats, especially in fish liver oils, while the storage fats of a majority of animals may be completely devoid of this vitamin. In contradistinction to the distribution of vitamin A, that of the carotenoids (some of which are provitamins A), is almost entirely reversed. Although both α- and β-carotene are sometimes found in animal fats,[331], [332] including butterfat,[333] and another provitamin A, cryptoxanthin, has been demonstrated in egg yolk[334] and in butter,[335] the provitamins are more frequently found in vegetable oils than in animal fats. For example, palm oil is an excellent commercial source of β-carotene. Vitamins D are found exclusively in animal fats although, when present, they occur in minimal quantities except in some fish liver oils. On the other hand, the tocopherols (vitamin E) have a much wider distribution, and occur in greater amounts, in the vegetable oils than they do in the animal fats. The same statement can be made concerning the occurrence and distribution of the so-called essential unsaturated fatty acids.

2. *The comparative amounts of vitamins and of essential fatty acids in animal and vegetable fats*

a. Vitamin A and provitamin A

Butter is the commonest source of vitamin A among the animal foods. According to a recent co-operative study,[336] the average vitamin A content of butter produced in the United States somewhat exceeds 15,000 I.U. per pound, although it is recognized that the quantity of vitamin A varies with season,

breed of the animals, and their diet. The highest concentration of vitamin A in any animal tissues occurs in the livers of certain fishes. Cod-liver oil (*Gadus morrhua*), which has always been regarded as a standard source of this component, has only a relatively low concentration, namely 600 I.U. per gm. The liver oils from a number of fishes such as the Atlantic mackerel (*Scomber scombrus*), barracuda (*Sphyraena argentea*), yellowtail (*Seriola dorsalis*), and the yellowfin tuna (*Neothunnus macropterus*) contain between 30,000 and 80,000 I.U. per gm.[337] Even higher quantities of vitamin A are found in other fish liver oils. These include bonito (*Sarda chilensis*), 120,000 I.U.; cabrilla pinta (*Epinephelus analogus*), 170,000 I.U.; swordfish (*Xiphias gladius*), 250,000 I.U.; ishinagi (*Stereolepis ishinagi*), 300,000 I.U.; black sea bass (*Stereolepis gigas*), 600,000 I.U.; and several varieties of shark, of which the soupfin (*Galeorhinus zyopterus*) has been reported as containing 640,000 I.U.,[338] and in one case 800,000 I.U.[339]

In addition to its occurrence in the liver of fishes and other animals, this vitamin is present in egg-yolk, in lung tissue, and in kidney tissue. Some may also be present in corpora lutea, although it is probably chiefly in the form of carotene. The retina, which has long been recognized as the site in which an exceedingly high concentration of a vitamin A-like compound occurs, is now known to contain vitamin A aldehyde.

Although the plants are the principal source of the carotenoids, these provitamins frequently occur there with a minimum amount of fat. Such is the case with turnip greens, spinach, and broccoli, as well as with such yellow, orange, or red-coloured foods as squash, carrots, apricots, persimmons, and tomatoes. On the other hand, of the commercial vegetable oils, red palm oil has the highest β-carotene content. Buckley[340] has recorded values as high as 1900 I.U. per gm in the oil of the fresh fruit, while Kaufmann[341] has noted a value of 3000 I.U.

Vitamin A occurs in margarine fat both as preformed vitamin A and as the provitamin. In the case of the United States, the average vitamin A content in margarine was formerly set at 9000 I.U. per lb (20 I.U. per gm). This figure was established as the standard for fortification of margarine by the Food and Drug Administration, in 1941, when it was believed that this value represented the average vitamin A content of butter.[342] However, since the higher figure for the vitamin A level in butter has been published,[336] practically all margarine produced in the United States contains this higher level of vitamin A (15,000 I.U. per lb or 33 I.U. per gm). Since legislation has been passed repealing the restrictions on the sale of coloured margarine, some varieties of margarine now sold contain carotene. Such sources of provitamins A may now be included as a portion of the vitamin A declared on the label of the product.[343] It has recently been demonstrated that margarine is an especially effective vehicle for facilitating the utilization of β-carotene by the rat.[172]

b. Vitamin D

Vitamin D is only rarely found in common animal fats, and never in vegetable fats. Small amounts may be present in butter. On the other hand, the most

concentrated sources of this vitamin are certain fish liver oils which have been widely employed as practical sources for therapeutic use. The values for the vitamin D in fish liver oils vary from 0 I.U. in sturgeon liver oil to approximately 40,000 I.U. per gm in the liver oil of the bluefin tuna (*Thunnus thynnus*). Liver oils such as those from the swordfish (*Xiphias gladius*) and from the yellowfin tuna (*Neothunnus macropterus*), contain approximately 10,000 I.U. per gm, while cod-liver oil (*Gadus morrhua*), long regarded as the standard medicine for rickets, contains only 100 I.U. per gm. Among the vitamin D-containing oils listed by BILLS[344] in decreasing order of their vitamin potency, cod-liver oil holds twenty-third place.

Since it has recently been demonstrated that vitamins D can be formed by irradiation of the provitamins D which occur in foodstuffs, this procedure has become widespread; consequently the availability of the vitamin in the foods has been increased. Thus, vitamin D is formed not only by irradiation of 7-dehydrocholesterol in animal fats but also by similar treatment of the ergosterol and dihydroergosterol in yeast, as well as of the dihydrositosterol in numerous vegetable fats.

c. Tocopherols (vitamins E)

Both animal and vegetable fats are sources of tocopherols. The most effective source of these compounds is wheat germ oil, which contains as much as 550 mg per 100 gm.[345] This quantity is more than 20 times that of the most concentrated animal source, namely cod-liver oil, which contains a maximum of 26 mg per 100 gm.[346] The comparative amounts of tocopherols present in some common vegetable and animal fats are listed in Table 28.

The comparative activity of oils from the standpoint of their physiological action may vary according to the distribution of the tocopherols. From the

Table 28. The tocopherol (vitamin E) content of some vegetable and animal fats

Oil or fat	*Tocopherol*	*Oil or fat*	*Tocopherol*
	mg/100gm		mg/100 gm
Vegetable fats:		Rice bran, refined[345]	90
Babassu, crude[345]	3	Safflower, crude[345]	80
Castor[345]	50	Sesame, refined[345], [347]	50, 5
Coconut, refined[345]	3	Soybean, crude[346]	212, 152
Coconut, hydrogenated[346]	3	Soybean, refined[345], [348]	175–110
Corn, crude[346]	119	Soybean phosphatide[345]	200
Corn, refined[345]	95	Wheat germ, crude[346], [347]	400–310, 520
Cottonseed, Wesson[346]	110	Wheat germ, solvent extracted[345]	550
Cottonseed, refined[345]	90		
Linseed[347]	23	*Animal fats:*	
Olive[345–347]	20, 25, 8	Cod-liver oil[346]	26
Palm, crude[345]	50	Lard, prime steam[345], [349]	2·8–0·5, 0·5
Peanut, crude[345]	52		
Peanut, refined[345]	48	Mangona shark liver oil[350]	10
Pecan, refined[345]	45	Oleo oil[345]	2
Rice bran, crude[345]	100	Soupfin shark liver oil[350]	4

standpoint of antisterility effect (which is generally considered to be the true vitamin E effect), α-tocopherol (5,7,8-trimethyltocol), β-tocopherol (5,8-dimethyltocol), γ-tocopherol (7,8-dimethyltocol), and δ-tocopherol (8 methyltocol) are effective in decreasing order. On the other hand, in terms of their antioxidant action (which is referred to as the tocopherol effect), the activity is in reverse order, being greatest for the δ-compound and least for the α-isomer. The proportion of the several component tocopherols present in any oil will obviously determine its antisterility potency or its antioxidant activity.

Although it is not certain that vitamin E plays a role as an antisterility agent in the human subject, it most certainly has other important functions in man. It is of great practical importance in the protection of fats from oxidation; the relatively greater resistance of the vegetable oils to rancidity as compared with animal fats is a reflection of the variation in tocopherol content. Moreover, the relatively low tocopherol content of olive oil renders it one of the vegetable fats most susceptible to oxidation. Vitamin E is apparently stable to the hydrogenation process;[351] in fact, a number of workers[352–354] have considered that hydrogenated fats are more reliable sources of tocopherols than is the unhydrogenated product.

d. Unsaturated fatty acids

The vegetable fats in general have much higher proportions of the so-called "essential" acids than do the animal fats (see Table 2, p. 108). Linoleic and linolenic acids are the only biologically active unsaturated acids in vegetable fats. Animal fats may include not only linoleic and linolenic acids, but also arachidonic acid, which exhibits the greatest biopotency of the 3 acids.

3. *The comparative digestibility and absorption of vegetable and animal fats*

Digestibility and absorption are important physiological indices of nutrition. No differences in the behaviour of vegetable *vs.* animal fat can be demonstrated in either of these functions. Thus, the coefficients of digestibility of 34 vegetable fats melting below 50°C varied between 94 and 99, with the exception of avocado fat (88) and teaseed oil (91). On the other hand, the variations in digestibility of 18 animal fats were between 93 and 99 (see Table 16, p. 147). Moreover, there was no apparent difference in digestibility of the higher-melting fats, which were more difficultly absorbed irrespective of whether they were of vegetable or of animal origin (Table 17, p. 148 and Table 18, p. 150). Finally, no appreciable differences in the rates of absorption were observed between vegetable and animal fats. From the standpoint of utilization, it would appear that animal and vegetable fats are of equal nutritional value.

4. *Growth tests with vegetable and animal fats*

Growth is the physiological response which has most often been accepted as an index of nutritional value. In a large number of studies for the evaluation of the nutritional value of vegetable and animal fats this index has been employed.

a. Experiments with normal rats

In our earlier discussion in this review, it was pointed out that greater weight gains obtain in animals which receive a fat-containing diet than in animals in whose case a fat-free regimen is employed. The question is a pertinent one as to whether or not vegetable and animal fats can function equally well in supplying this need.

The chief controversy has been as to whether or not butterfat possesses a specific growth-promoting effect. On the positive side of the argument, SCHANTZ, ELVEHJEM, and HART[355] reported that weanling rats grew better over a 6-weeks period when they were fed on liquid skimmed milk homogenized with butterfat than when corn oil, cottonseed oil, coconut oil, or soybean oil was homogenized into the fat. The differences in growth-promoting power of the fats largely disappeared after the 6-weeks period following weaning. It was later reported[356], [357] that the saturated fatty acid fraction of butter was more effective in producing growth than was a comparable amount of the whole butter, while the volatile and unsaturated fatty acid fractions were ineffective. The unsaturated acid fraction served effectively for growth after hydrogenation.[357] It was concluded that the superior growth on butterfat observed in the earlier experiments[355] was to be ascribed to the presence of certain long-chain saturated acids which were present in butterfat but not in other fats. However, two independent groups of investigators[358], [359] have failed to confirm the finding that the saturated fatty acid fraction of butter possesses any specific effect on growth.

GEYER *et al.*[360] also reported a superior growth-promoting action on the part of the liquid portion of butter which remained after the separation of the solid glycerides in acetone solution at — 4°C, in one experiment. In a repetition of the test, they failed to confirm the original findings. JACK and HINSHAW[361] likewise noted better growth on the liquid fraction of butter than on other fractions, provided care was taken to prevent oxidative destruction. It is obvious that the earlier reports of SCHANTZ *et al.*,[355], [356] in which the superior effect of butterfat on growth is ascribed to saturated rather than to unsaturated acids, and the later studies in which it was traced to the unsaturated acids,[360], [361] are contradictory. Moreover, the results obtained by DEUEL and collaborators[362] on whole butter do not bear out this finding.

BOER[363] also reported superior growth in rats when butterfat was fed, as contrasted with results obtained with olive oil. However, the choice of the vegetable oil was an unfortunate one, since this fat contains very little tocopherol and consequently readily becomes rancid. DEUEL and co-workers[362] likewise found that the weight gains of rats fed an olive oil sample which had become rancid were inferior to those observed when other vegetable oils or butterfats were employed. BOER and collaborators[284] reported that rapeseed oil was inferior to butterfat in promoting growth in rats. This finding has been confirmed by DEUEL and associates[364] as well as by EULER, EULER, and LINDEMAN.[365] The low growth-promoting ability of rapeseed oil has been correlated with its low digestibility in the rat.[281]

In contradistinction to the positive results obtained with butterfat, a number of negative reports fail to confirm the hypothesis that the growth-promoting action of butterfat is superior to that of vegetable fats. Thus, DEUEL and co-workers[362] reported that no superior growth could be observed at any period over 12 weeks following weaning, in male or female rats fed butter-containing diets, as compared with rations containing corn, cottonseed, olive, peanut, or soybean oils or a margarine fat. In these tests, dried skimmed milk powder was employed as a vehicle for the fat, instead of liquid skimmed milk. The fact that the gains-in-weight in all cases represented true growth was confirmed by the determination of the rate of bone growth. In addition to using the gain-in-weight as a comparative index, it was also established that a similarly efficient utilization of the several diets obtained. Moreover, analyses of the tissues of the rats raised on the several dietary regimens indicated a similar distribution of protein, fat, carbohydrate, and ash.[245] One is therefore forced to the conclusion that the extent of growth obtained by means of the several vegetable fats is identical with that of the rats receiving butterfat.

Several suggestions have been proposed to account for the better growth observed by SCHANTZ *et al.*[355] on the butter diets than in cases in which other fats were fed. In the first place, there is a possibility that a greater food consumption may have been responsible, since it was shown that rats prefer a diet of bland fat flavoured with diacetyl (butter flavour) to one which is unflavoured.[366] In the tests on vegetable fats by DEUEL *et al.*,[362] cited above, butter flavour was used; this probably accounts for the uniformly high food consumption in the several dietary groups. BOUTWELL and collaborators[367] included diacetyl with the corn oil. Although they failed to improve the food consumption sufficiently to cause growth identical with that in the butterfat tests, there was an appreciable increase in food consumption as a result of adding this flavouring.

EULER, EULER, and SÄBERG[368] likewise gave a negative answer to the question as to whether or not butterfat has a specific growth-promoting property. These workers noted better growth when oleomargarine was the dietary fat than when butter served in that capacity. More recently, EULER *et al.*[369] have extended their studies on growth with butter and margarine diets over a period of 700 days; growth was equally satisfactory with the two fats over the entire period. Other workers, who have demonstrated a similar growth-promoting behaviour of vegetable fats and of butter, include DEUEL *et al.*,[362], [370] ZIALCITA and MITCHELL,[371] HENRY and co-workers,[358] and LASSEN and BACON.[372]

(*a*) *The effect of vaccenic acid on growth*—Several suggestions have been made as to the reason for the greater growth of rats on butterfat diets as compared with those containing vegetable fats. For example, vaccenic acid, 11 octadecenoic acid, has recently been cited as the acid in butterfat responsible for the superior nutritional value of this fat. BOER, JANSEN, and KENTIE[284] noted that summer butter contains appreciable amounts of this unsaturated acid, while it is absent from winter butter, as well as from vegetable fats. The presence of the acid in

butterfat and in animal fats has been confirmed by a number of workers,[373–375] while GEYER *et al.*[375] demonstrated its absence in vegetable fats. However, hydrogenated vegetable fats have been shown to contain relatively large amounts of vaccenic acid.[373], [374]

The first report of the high nutritional value of vaccenic acid was that of BOER *et al.*;[376] these workers found that the growth of rats on a rapeseed oil diet was increased if the rations were supplemented with vaccenic acid prepared from China wood oil. It was further suggested that the failure of the EULER group[368], [377] to confirm the earlier report of BOER[363] and of BOER and JANSEN[378] on the superiority of butterfat over margarine or olive oil could be attributed to the probable lack of vaccenic acid in the winter butter produced in Sweden.

However, a number of reports have failed to confirm the fact that vaccenic acid is a dietary adjunct. Thus, DEUEL and associates,[364] using the same procedure as BOER and co-workers,[284] confirmed the fact that rapeseed oil produced a poorer growth than did cottonseed oil. However, the addition of vaccenic acid to either the rapeseed oil or the cottonseed oil diets did not augment growth. Similar negative results have been recorded by EULER *et al.*,[365] as well as by NATH, BARKI, ELVEHJEM, and HART.[379] In fact, BOER and collaborators[380] have recently retracted their earlier statement.

It is possible that vaccenic acid may have been mistaken by BOER *et al.*[376] for 12-octadecenoic acid, since these two unsaturated acids have quite similar properties. However, investigations on the nutritional value of this acid, by DEUEL *et al.*,[370] have failed to demonstrate any specific beneficial effects on the part of the latter acid. It is therefore apparent that there is no convincing evidence that vaccenic acid is specifically required by the animal organism.

(*b*) *Experiments on prematurely-weaned rats*—In their original report on the superior nutritional value of milk fats as contrasted with vegetable fats, BOUTWELL and associates[357] noted that the need for the particular fatty acids present in butterfat could no longer be demonstrated if the animals were kept on the stock diet until they were 30 days of age, instead of being weaned and placed on the test diets at the age of 21 days. These investigators further stated that this need for butter was accentuated in rats placed on the test diets at 14 days of age rather than at the usual 21-day period. However, tests in the author's laboratory[381] indicated that such prematurely-weaned rats grew as rapidly on complete diets containing corn, cottonseed, olive, peanut, or soybean oil, or a vegetable margarine fat, as on one containing a butterfat. Moreover, ZIALCITA and MITCHELL[371] were able to raise rats as effectively on corn oil as on a butterfat when the animals were weaned at the age of 7 days and were continued thereafter on synthetic diets. The evidence would therefore tend to discount the age factor as regards the requirement for butterfat.

b. Comparative effectiveness in animals receiving restricted diets

Another procedure for the evaluation of the nutritional merit of a foodstuff is to compare the results when a restricted diet is employed with those when the

foodstuff to be tested is fed. Under such conditions, if a specific fat is required for growth, the need for it may not be satisfied during periods of restricted food intake. However, it was found that the rate of growth of rats on diets containing several vegetable oils or a vegetable margarine fat, when fed at such a level that growth occurred at a subnormal rate, was similar to that obtained on a diet containing a butterfat.[382] During a subsequent period, when the same diets were given *ad libitum*, the response of the rats to the vegetable fat diets was similar to that of the animals on the butterfat regimen.

c. Experiments on rats receiving growth hormone

A procedure for nutritional evaluation, which is the converse of that in which restricted feeding is employed, involves the determination of whether or not the diets under investigation will be satisfactory at abnormally high levels of growth. It is conceivable that a substance might be satisfactory for growth under normal conditions but that it might fail under conditions of stress caused by a high rate of growth. As an example of the latter, ERSHOFF and DEUEL[383] reported that the lack of vitamin A can be accentuated when the growth hormone is injected into rats on a vitamin A-free diet. In their experiments, the symptoms of avitaminosis A appeared sooner, and an earlier death resulted under such conditions. When an active pituitary growth hormone was injected into rats, it was found that diets containing corn, cottonseed, olive, peanut, or soybean oils, or a margarine fat, supported the increased growth as effectively as did one containing butterfat.[382]

d. Growth and nutrition experiments on children

LEICHENGER, EISENBERG, and CARLSON[384] have recently reported the results of a two-year test carried out on children in two orphanages. In one group, margarine served exclusively as the table spread while, in the second case, a comparable diet was used except that butter replaced the margarine. It was found that the well-being of the children during the test, and their growth during the two-year period, were similar in the two groups. It would therefore appear that, provided the nutritional identity of the butterfat diet and the diets containing vegetable fats is established, the results on rats are also applicable to man.

5. Pregnancy and lactation performance of rats on vegetable or animal fats

Other nutritional indices which have been used to compare vegetable and animal fats include their efficiency in animals during pregnancy and lactation. As indicated earlier, growth, pregnancy, and lactation afford progressively more critical methods for evaluation of the nutritional value of foodstuffs.

It is a well-known fact that fat-low or fat-free diets are especially unsatisfactory for lactation.[385], [386] However, SURE[387] found that satisfactory lactation could not be produced on otherwise *inadequate* diets by butterfat, lard, hydrogenated oil, olive oil, or wheat germ oil. On the other hand, MAYNARD and RASMUSSEN[388] reported that, on *adequate* diets, improvement in lactation

obtained in rats when corn or coconut oil was added to a fat-free diet, although hydrogenated coconut oil was found to be ineffective.[389]

There is some question as to what factor in fat is responsible for the beneficial effect of this foodstuff on milk production. According to QUACKENBUSH *et al.*,[104] linoleic acid is necessary for satisfactory lactation. If this is true, vegetable fats should be preferable for this function since, in general, they contain more essential fatty acids than do the animal fats. This would also explain the findings of LOOSLI and associates[389] that hydrogenated coconut oil is ineffective. On the other hand, these latter workers[389] were unable to demonstrate an improvement in lactation of rats on a fat-free diet when 125 mg of ethyl linoleate were administered daily.

Irrespective of what causes improved lactation when fat is fed, there is evidence of the equivalence of vegetable and animal fats in effecting this phenomenon. Thus, DEUEL *et al.*[390] reported that rats fed on diets containing a series of vegetable oils, or a vegetable margarine fat, presented essentially the same reproductive capacity and the same increase in lactation as did animals on a butterfat regimen. These data are summarized in Table 29.

Table 29. Comparative nutritional value of vegetable oils, a vegetable fat, margarine and butterfat as indicated by pregnancy and lactation tests[390]

Skimmed milk powder mixed with one of the following fats	*Pregnancy tests*			*Lacation tests*	
	Animals bred	*Litters cast*	*Average 3-day weight per rat*	*Rats died before weaning at 21 days*	*Average 21-day weight per rat**
			gm		gm
Butterfat	29	29	9·09	23	33·4 (84)
Corn oil	31	28	9·06	1	32·9 (112)
Cottonseed oil	31	30	9·15	2	34·4 (98)
Margarine fat	35	33	7·57	8	34·8 (112)
Olive oil	21	18	7·88	1	35·2 (70)
Peanut oil	32	31	8·03	4	33·6 (84)
Soybean oil	28	26	7·60	1	34·5 (98)

* Figures in parentheses indicate the number of rats weaned. Only seven-rat litters included in averages.

EULER, EULER, and RÖNNESTAM-SÄBERG[369] have reported an experiment carried out over 18 months which indicates a marked superiority of margarine fat over butterfat as demonstrated by reproduction tests. The total weight at 28 days of all offspring produced over the year and one-half period was 8508 gm for the group on the butterfat diet as compared with 15,956 gm for those on the margarine fat regimen. Although the supplementation with vitamin E improved the response of the butterfat group in a subsequent series of tests, the weights of the supplemented butterfat group did not equal that of the unsupplemented margarine group. The results obtained from pregnancy and lactation tests do not indicate any nutritional response to animal fats which is not shared by the vegetable fat group.

6. *Growth and reproduction of rats over many generations on a diet containing a vegetable margarine*

One procedure for estimating the nutritional value of foodstuffs which combines growth, pregnancy, and lactation tests is the so-called "multigeneration" method. Much more information can be gleaned from a test of this kind carried out over a number of generations than can be obtained from growth, pregnancy, or lactation studies on a single generation. It is conceivable that a diet which would be satisfactory for growth or even for reproduction and lactation during one generation might be unsatisfactory after several generations, over which deficiencies could develop.

SHERMAN and CAMPBELL[391], [392] devised a simple diet which allowed rats to continue growth, reproduction, and lactation and which at the last report had progressed successfully to the sixty-seventh generation, and was still continuing.[393] The diet "A" which was employed to the fortieth rat generation by these workers consisted of whole milk powder (1/6) and ground whole wheat (5/6), plus NaCl to the extent of 2% of the weight of the wheat. The "B" diet employed after the fortieth generation contained one-third whole milk powder and two-thirds ground whole wheat with 2% of sodium chloride. SHERMAN and CAMPBELL[394] reported that the life span of the animals on diet B was prolonged significantly over that of the rats on diet A.

A somewhat comparable test has been under way in the author's laboratory since April, 1940, during which the rats have received the same diet as diet B used by SHERMAN and CAMPBELL, except that skimmed milk powder containing margarine fat in an amount comparable to the butterfat in whole milk powder was employed. At the end of 10 generations, the rate of growth of the male and female rats was considerably higher than at the start of the test.[395] Moreover, fertility and lactation have been maintained at a high level over the entire period. In a later report,[396] after 25 generations, it was shown that the same high level of growth and reproduction had continued. At the present time (July, 1953), the experiment has successfully progressed to the thirty-seventh generation. These results offer cogent evidence of the equivalence of butterfat and of vegetable margarine fat when tested by the most rigorous experimental procedure, namely, that of the multigeneration experiment.

5. The Comparative Nutritive Value of Mono-, Di-, and Triglycerides

Although it is generally agreed that mono- and diglycerides are intermediates in fat digestion and in fat absorption, as well as in its intermediary metabolism, no evidence as to their nutritional value has been available until recently. BRAUN and SHREWSBURY[397] were the first to report on this factor. They noted that monostearin and monolinolein were nutritionally approximately equivalent to lard in producing growth in rats, when fed at a level of 8%. AMES and coworkers[398] reported no difference in growth, reproduction, or lactation performance between rats fed mono- or triglycerides prepared from cottonseed oil, and those fed the refined cottonseed oil. The monoglycerides were isolated by molecular distillation. More recently, MATTSON *et al.*[399] have investigated the

mono-, di-, and triglycerides isolated from soybean oil and from coconut oil, as well as the purified mono- and triglycerides of oleic, stearic, and lauric acids. These authors concluded that mono-, di-, and triglycerides of corresponding fatty acid composition were nutritionally equivalent. The caloric efficiencies of the mono- and triglycerides of both stearic and lauric acids were found to be low. These workers attributed this fact entirely or partially to poor absorption. In later investigations, MATTSON and associates[400] isolated and identified, by unequivocal methods, as much as 16% of monoglyceride and 36% of diglyceride in the total lipids separated from the lumen of the intestine of rats, following the feeding of triglyceride. This supports the work of FRAZER and SAMMONS,[401] who previously interpreted the high hydroxyl value of the lipids isolated from the lumen of the intestine as indicative of the presence of monoglycerides.

6. The Influence of the Type of Fat on the Toxicity of Alloxan

HOUSSAY and MARTÍNEZ[402] were the first to point out that the toxic and diabetogenic action of alloxan may be considerably influenced by dietary factors. Thus, in the white rat, low-protein and high-lard diets increased the toxicity of alloxan (recorded as mortality during 10 days following the injection of the drug), while high-protein and high-coconut oil diets had a protective effect. On the other hand, GYÖRGY and ROSE[403] reported a high toxicity in rats, as demonstrated by early mortality (within 2 days) and haemoglobinuria, on diets free from yeast and high in lard or coconut oil. The early mortality was significantly reduced by the inclusion of yeast or tocopherol in the diet. Moreover, tocopherol was found to prevent the development of haemoglobinuria caused by alloxan.

In a re-evaluation of this problem, RODRIGUEZ and KREHL[404] confirmed the increased toxicity occasioned by low-protein diets in alloxan poisoning. On the other hand, it was found that the inclusion of coconut oil or of such short-chain acids as caprylic acid in the diet resulted in a marked decrease in the diabetogenic action of alloxan. In contradistinction to these results, it was shown that diets containing large amounts of lard produced a high incidence of diabetes, as well as a high mortality. The results were not influenced by tocopherol, in contrast to those of GYÖRGY and ROSE.[403] Strangely enough, the results on palmitic acid showed the lowest mortality and the lowest incidence of diabetes of any of the fats examined. The divergent behaviour of coconut oil and lard is believed to be due to a difference between the method of metabolism of a fat composed primarily of long-chain acids (lard) and one containing a large proportion of short-chain acids (coconut oil).

V. Possible Deleterious Effects of Fat in the Diet

1. Cholesterol Deposition and Fat Intake

One of the most serious criticisms which has been levelled against the inclusion of fat in the diet is the charge that its continued ingestion results in increased levels of body cholesterol. Animal fats have been considered to be the more serious offenders, because they contain preformed cholesterol, while vegetable

fats are less harmful in that the phytosterols in these products cannot be absorbed or changed to cholesterol,[405–408] even when fed with bile salts.[408]

However, it is believed that fat *per se* also contributes to the production of cholesterol, which is formed synthetically in the animal body. Acetic acid, related to the two-carbon fragment, which is believed to be formed during the metabolism of fat, has been cited as the precursor of cholesterol. Under such conditions, animal and vegetable fats would share equally in furnishing the building stones for the synthesis of cholesterol. While there is adequate evidence to prove that cholesterol can originate from acetic acid,[409] one should not forget that acetic acid may also arise directly from carbohydrate, as well as from fatty acids. Moreover, it can be formed indirectly from this carbohydrate by breakdown of the body fat originally synthesized from this foodstuff. ALFIN-SLATER and her collaborators[410] have recently shown that the rate at which cholesterol is synthesized in rats is not influenced by diet; as rapid an increase in deuterocholesterol obtained in the blood and liver of rats on a fat-free diet as in animals receiving a 30% fat regimen.

2. Toxic Products From Heated Fats

It has already been pointed out that one of the factors which influence digestibility of fats is polymerization. When fats are heated to 275°C or higher, a polymer formation takes place. According to LASSEN, BACON, and DUNN[282] and ROY,[235] the coefficients of digestibility of such fats are decreased in proportion to the extent of polymerization which has occurred.

CRAMPTON and MILLAR,[411] in 1946, were the first to observe that the feeding of linseed oil previously heated at 275°C, in the presence of inert gas, resulted in a high death rate. Several other workers[412], [413] had previously reported deleterious effects of fats which had been subjected to high temperatures. In some cases, paralysis resulted similar to that described by MACKENZIE *et al.*[414] as occurring in vitamin E deficiency. However, it must be considered that the oils used by these workers had in all cases been subjected to heating in the presence of air, resulting in oxidation. CRAMPTON and his associates[415] later showed that a decrease in weight gains per 1000 calories ingested occurred in rats when the diets contained corn, herring, linseed, peanut, rapeseed, or soybean oil which had been subjected to heating at 275°C anaerobically. A marked effect was noted for peanut oil, in which the gain-in-weight per 1000 calories ingested decreased from 87 for the unheated oil to 30 for the oil heated at the above temperature for 30 hr. In the case of linseed oil, the gain-in-weight amounted to only 4 gm per 1000 calories for the oil heated for 12 hr, as contrasted with a value of 80 for the control values for this fat. On the basis of later work from this same laboratory,[416] it was suggested that the primary cause of the reduced nutritive value was the presence of one or more dimeric fatty acid radicals; these are believed to act in some way inimical to the well-being of the animals. Still more recently, CRAMPTON *et al.*[416a] concluded that the toxicity of heated fats may result from the formation of cyclized or branched-chain acids.

Although polymerization results when fats are heated at 275°C, there is no

evidence which can be detected by nutritional tests that the temperatures to which fat is ordinarily subjected in cooking cause injury to the fat. Roy[295] reported no decrease in digestibility in several fats subjected to 250°C. Likewise, Deuel *et al.*[417] found that margarine fat, which had been heated at 205°–210°C for a long period during which a number of batches of potato chips were prepared in it, gave as effective growth in young rats, when incorporated in their diet to the extent of 40%, as did unheated oil.

3. Toxicity of Rancid Fats

The effects of rancid fats and products of fat oxidation has been discussed in the chapter on autoxidation of fats and related substances, page 51.

4. Miscellaneous Conditions in which Fat Ingestion is Contraindicated

According to Li and Freeman,[418] a greater degree of leucopaenia was noted in rats subjected to benzene when they were maintained on a high-fat diet than when a low-fat diet was fed. The leucocyte count was reduced, irrespective of the protein level in the diet.

A marked haemolytic anemia has been demonstrated by Davis and Gross[419] in dogs, as a result of the daily administration of 60 gm of fat and of 10 mg per kg of choline. This was accompanied by significant elevation of the icteric index. A somewhat similar condition was shown to obtain in two human subjects who ingested ¼ lb of butter and 400 mg of choline for breakfast; the depression in the red blood cell count continued for 36 hr, after which a reticulocytosis occurred.

McLean and Beveridge[420] have noted that increasing levels of fat in the diet augmented the extent of liver necrosis in rats on a basal necrogenic diet. On the other hand, the carbohydrate level of the diet was without effect on this condition.

VI. Optimum Levels of Fat in the Diet

1. Conclusions Based upon Experiments on Rats

The beneficial effects of fat in the diet are so varied in character as to cause one to question whether fats are only optional components of the rations or whether they should be regarded as obligatory constituents of the diet.

The figure at which the optimum fat level is established depends to some extent upon the method of evaluation. By most of the methods employed in determining the nutritional value of foodstuffs, somewhat better results were obtained when the diet contained 20 to 40% of fat by weight. However, in many instances, the results obtained were quite satisfactory when only 10% of the diet consisted of fat.

One question which arises is whether or not all types of fat can fulfil the nutritional requirements equally well. At the present time, the data available are insufficient to answer this question. In the case of margarine fat and cottonseed oil, the best results were obtained on the 20% and 40% fat diets.

The determination of the optimum amount of fat as a source of essential fatty

acids leads to different results, depending upon the particular situation involved. The requirement for the essential acids for growth varies with sex, being higher with males than with females. In the former case, it probably exceeds 200 mg per day, while in the latter instance the figure is about 50 mg daily. On the other hand, when the essential acids serve as protective agents against x-radiation injury, amounts as small as 10 mg per day have been shown to be effective in rats; however, the ability to withstand x-radiation injury was found to be progressively increased when 10, 20, 50 and 100 mg of linoleate was given daily.[421]

It seems probable that the beneficial effects of fats are not to be traced exclusively to their essential fatty acid content. One experiment,[9] which indicates this fact, was carried out with female rats previously depleted of fat. After the administration of 20 mg of linoleate daily over a number of weeks until a plateau in body weight was reached, the dosage was increased to 60 mg without causing any further increase in weight. However, when 10% of cottonseed oil was added to the diet, a marked growth-stimulating effect obtained, which continued over a number of weeks. These results led to the conclusion that fat possesses some beneficial action not traceable to the essential fatty acids or tocopherol which it contains.

2. Conclusions Based upon Experiments on Man

It is difficult to determine how far the results obtained with rats can be translated into terms of human nutrition. According to BRANDT,[422] the daily intake of fat by the British increased from 99 gm per day before World War I to a figure of 124 gm by 1934. A similar fat consumption also obtained for the Americans and Germans during the latter period; fat accounted for 30% of the calories in their dietaries at that time. In sharp contrast to this relatively high intake by the Occidentals, the intake for Orientals has been shown to be quite low. According to BRANDT,[422] the Japanese consumed a diet before the war containing only 6 to 10% of fat calories. Moreover, SHEN[423] estimates that the soldiers of South China in World War II ate fat to the amount of only 3% of their caloric intake, which is less than one-tenth of the 40% which HOWE[424] calculates as the average consumption of the American soldiers in World War II.

The Food and Nutrition Board of the National Research Council (United States)[425] has made the following suggestions as to the human requirement for fat:

> FAT. There is available little information concerning the human requirement for fat. Fat allowances must be based at present more on food habits than on physiological requirements. While a requirement for certain unsaturated fatty acids (the linoleic and arachidonic acids of natural fats) has been amply demonstrated with experimental animals, the human need for these fatty acids is not known. In spite of the paucity of information on this subject, there are several factors which make it desirable (1) that fat be included in the diet to the extent of at least *20 to 25 per cent* of the total calories and (2) that the fat intake include essential unsaturated fatty acids

to the extent of at least 1 per cent of the total calories. At higher levels of energy expenditure, e.g. for a very active person consuming *4500 calories* and for children and for adolescent persons, it is desirable that *30 to 35 per cent* of the total calories be derived from fat. Since foodstuffs such as meat, cheese, nuts, etc., contribute fat to the diet, it is necessary to use separated or "visible" fats such as butter, oleomargarine, lard, or shortenings to supply only one-third to one-half the amounts indicated.

Much experimental work is still required to determine what quantities are to be considered as the optimum for man. It is self-evident that the capacity for fat is limited and that, when this quantity is exceeded, gastric distress follows. It is of considerable importance to determine to what extent tolerance for fat varies with the type of fat and the method of preparation of the food into which it is incorporated.

VII. Concluding Remarks

The more modern concept of the role of fat in nutrition would seem to assign more importance to this foodstuff than was formerly the case. Instead of being regarded merely as a concentrated source of calories, this foodstuff is now considered to be an essential requirement for many physiological processes of the body. Thus, it possesses the capacity to spare protein, which is not shared by carbohydrates. Prefeeding with fat enables rats to adjust their metabolism in such a way as to extend their survival period during a subsequent fast far beyond that possible in rats prefed with other foodstuffs. The commanding role which fat plays in metabolism is demonstrated by such physiological phenomena as the rate of growth, pregnancy, lactation, and physical capacity. Moreover, the importance of fat in the diet can be demonstrated by its beneficial effects in such conditions of stress as thyrotoxicosis, x-radiation, cold, and partial hepatectomy.

Although the importance of fat can, in part, be ascribed to the essential unsaturated acids which it furnishes, it is believed that there are beneficial effects over and above that of the essential fatty acids. The whole field of the importance of fat in nutrition is one concerning which our information is still fragmentary. We can look forward to continued elucidation of this field in the future.

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4

THE SURFACE PROPERTIES OF FATTY ACIDS AND ALLIED SUBSTANCES

D. G. Dervichian

THE molecules of fats and of the derived fatty acids are relatively large and contain well-separated polar and paraffinic regions which behave quite differently from the point of view of the type of interaction they can exert. The peculiar surface properties of fats are mainly the consequences of this particular structure of their molecules. The study of the variation of surface and interfacial tension represents the most fruitful source of indirect information on surface structure, on interaction between two liquids and, above all, on adsorption which is properly a surface phenomenon. This explains why in most of the analyzed work measurement of surface and interfacial tension is the main technique.

Only results concerning fatty acids, their salts (soaps) and esters, triglycerides and phospholipids are reported. Data on detergents, such as alkyl sulphates, quaternary ammoniums, etc., have deliberately been omitted. This very important group of substances deserves a particular study, but their surface properties are, in the main, comparable to those of soaps which are described here.

I. THE SURFACE OF LIQUID AND SOLID FATTY SUBSTANCES

1. *Surface tension of fatty substances*

a. Fatty acids

The only systematic data on the surface tension γ of series of normal fatty acids at different temperatures is due to HUNTEN and MAAS.[1] The curves of Fig. 1 have been drawn mainly by making use of their results. The data for formic and acetic acid, given only for 15, 20, and 30°C, are taken from ROLAND and LEK.[2] The values for propionic acid determined by these authors correspond with those of HUNTEN and MAAS within the limits of accuracy of the measuring methods (i.e. one or two units of the first decimal). Values for oleic acid at 20°C and ricinoleic acid at 16°[3] are indicated on the same graph.

This figure shows that, with the exception of the C_8 and C_{10} acids, the different points fall on straight lines. But the slope of the different lines (i.e. the temperature coefficient $d\gamma/dT$ of the surface tension) varies with the acid. From a value of $-0{\cdot}11$ for formic and acetic acid, $d\gamma/dT$ decreases and reaches $-0{\cdot}076$ for stearic acid.

From the curves in Fig. 1 two sets of values can be extracted. One is relative to the surface tension at a given temperature and the other is relative to the respective temperatures at which the different acids have the same given value

for the surface tension. On Fig. 5 have been plotted the surface tensions of the fatty acids at 75°C against the number of carbon atoms. In 1949, WACHS, UMSTÄTTER, and REITSTÖTTER[4] gave values of the surface tension at 75°C of purified or synthesized normal fatty acids with both even and odd numbers of carbon atoms from C_{10} to C_{19}. The values given by these authors for the even acids correspond quite well with those derived from the measurements of HUNTEN and MAAS and have been plotted on the same curve (Fig. 5). The interesting point is that the surface tensions of the odd C numbered acids fall consistently on another curve, above that corresponding to the even acids. Thus the alternation which is well known for other properties (e.g. melting points) reappears with the surface tension. On Fig. 6 are plotted, against the number of

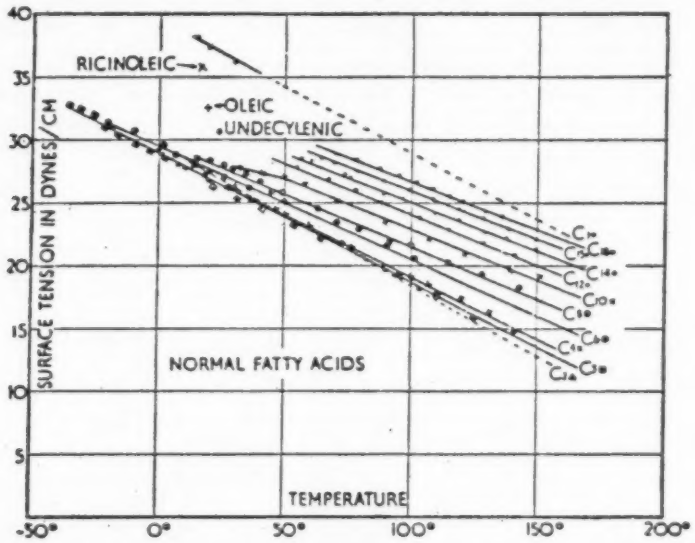

Fig. 1. Surface tensions at different temperatures of the normal fatty acids. Values for oleic, ricinoleic and undecylenic acids are shown for comparison.

carbon atoms in the molecule, the temperatures at which the same value of 25 dyne per cm is found as surface tension for the different fatty acids. These values are obtained by cutting the curves of Fig. 1 by the horizontal line $\gamma = 25$ dyne per cm.

The variation of both curves (Figs. 5 and 6) is consistent with the general idea that surface tension is related to cohesion forces. One curve (Fig. 5) indicates that the longer the acid chain the larger is the cohesion energy in the liquid and consequently the higher is the value of the surface tension. The other curve (Fig. 6) shows that the stronger the interaction between molecules, as a consequence of the number of carbon atoms, the higher must be the temperature (i.e. the antagonistic kinetic energy) in order to reduce the cohesion and consequently the surface tension to the same given value.

It can be seen in Fig. 1 that the surface tension value for oleic acid, which has been determined at 20° falls approximately on the extrapolation of the curve

corresponding to stearic acid. Thus oleic acid, which has a melting-point nearly equal to that of the saturated normal C_{10} acid, has a surface tension corresponding to that of the C_{18} acid of the same series. Therefore the chain length appears to be he governing factor. The fact that the value of γ for ricinoleic acid, which also has 18 C atoms, falls definitely above the values for oleic or stearic acid is e idently related to the existence of the functional alcohol group in its molecule. ? hese OH groups create between adjacent molecules much stronger interactions (probably hydrogen bonds) than the interaction between ordinary hydrocarbon chains. The same sort of reason must be given to explain the anomalous behaviour of formic acid whose values stand quite apart on Figs. 1, 5, and 6.

b. Esters of the fatty acids

Numerous and carefully established data can be found in one of the recent publications of VOGEL[5] on the surface tension at different temperatures of a

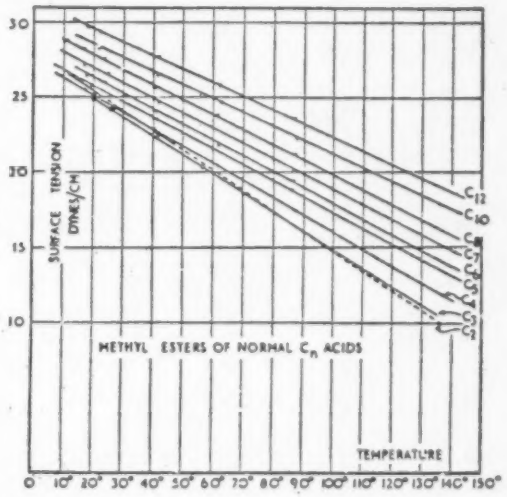

Fig. 2. Surface tensions at different temperatures of the methyl esters of the normal fatty acids.

great variety of alkyl esters. Part of these data has been used to draw the set of curves of Fig. 2, relative to the methyl esters, and of Fig. 3, relative to ethyl esters, of the normal fatty acids from C_1 to C_{12}. Like those for the fatty acids, the curves, representing the surface tension values for these esters as a function of the temperature, are straight lines. Here again the temperature coefficient $d\gamma/dT$ varies with the chain length, from $-0{\cdot}013$ for the shorter to $-0{\cdot}09$ for the longer.

Two other pairs of curves have been derived from the set of curves of Figs. 2 and 3 as was done with the fatty acids. One pair (Fig. 5) gives the surface tensions at 75°C of the methyl and ethyl esters. The other pair (Fig. 6) indicate the temperatures at which the same surface tension value of 25 dyne per cm is given by the different methyl and ethyl esters. The figures on the abscissae axis correspond to the number of carbon atoms in the esterified fatty acid. The same general remarks can be made, as in the case of the fatty acids, concerning the

increase of surface tension with chain length. There is a marked influence of chain configuration on the surface tension: the surface tensions of compounds with branched chains are lower than those with straight chains.

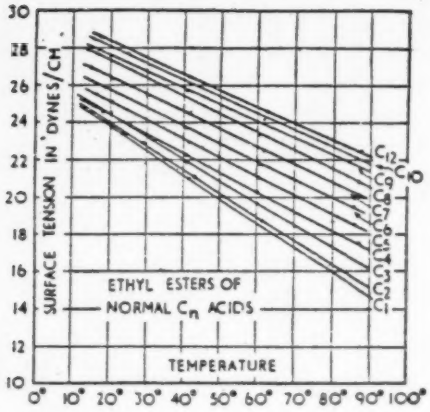

Fig. 3. Surface tensions at different temperatures of the ethyl esters of the normal fatty acids.

c. Triglycerides

The only comprehensive data on triglycerides are found in the old but careful work of JAEGER[6] who determined the surface tension of the series of triglycerides corresponding to the fatty acids from C_1 to C_{18} for a series of temperatures

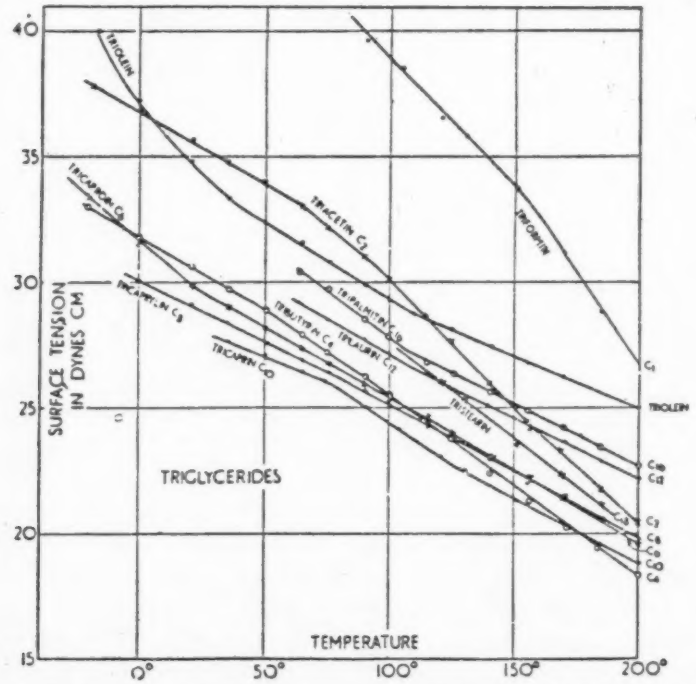

Fig. 4. Surface tensions at different temperatures of the series of triglycerides. The number of carbon atoms C_n refers to the fatty acid chain length.

starting below 0° and reaching 200°C. Previously to this work, WALDEN[7] had determined the surface tension of tripalmitin and tristearin in a range of temperature between *ca.* 55° and 120°. WALDEN's values are constantly lower than those of JAEGER by approximately 2 dyne per cm, but the shift between the values of the two triglycerides for a given temperature is in both cases of the same order. An examination of the values given by JAEGER for other substances at different temperatures shows that they coincide with the best known and generally

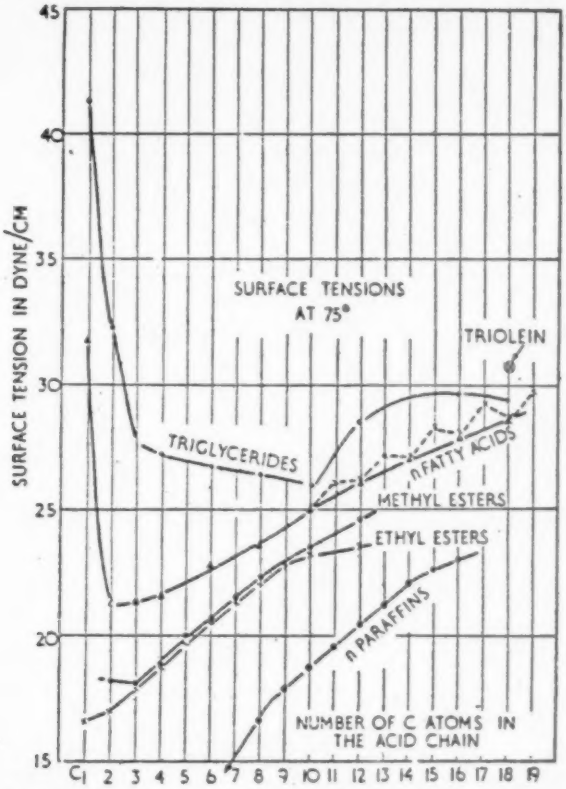

Fig. 5. Changes in surface tension as a function of the number of carbon atoms, measured at the same temperature (75°), for the triglycerides, fatty acids, esters and paraffins.

admitted recent values (e.g. water and benzene). On the contrary WALDEN's figures for a series of liquids are constantly below the admitted values. It should be noted, however, that measurements were made in 1929 by JOGLEKAR and WATSON[8] on five of the triglycerides (C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}) and that their values are intermediate between those of JAEGER and those of WALDEN. Unfortunately, these last measurements have been made within a rather narrow temperature range. These discrepancies are apparently due to the calibration of the instruments used. However, even if this casts some doubt on all the obtainable data (although JAEGER's results appear to be the more reliable), the relative place of the corresponding curves can still be taken into consideration, since the same instrument has been used through the set of measurements.

The curves of Fig. 4 have been plotted using JAEGER'S data. These curves show that the surface tension of triglycerides is far from being a linear function of the temperature. But the relation to the chain length appears very clearly in the two derived curves: one (Fig. 5) giving the surface tensions at a fixed temperature of 75°, and the other (Fig. 6) indicating the temperatures at which each of the triglycerides has a given surface tension of 25 dyne per cm. It can be seen that, in relation to the chain length the series of saturated triglycerides can be subdivided into three groups: (1) C_1 and C_2 which are quite distinct, having

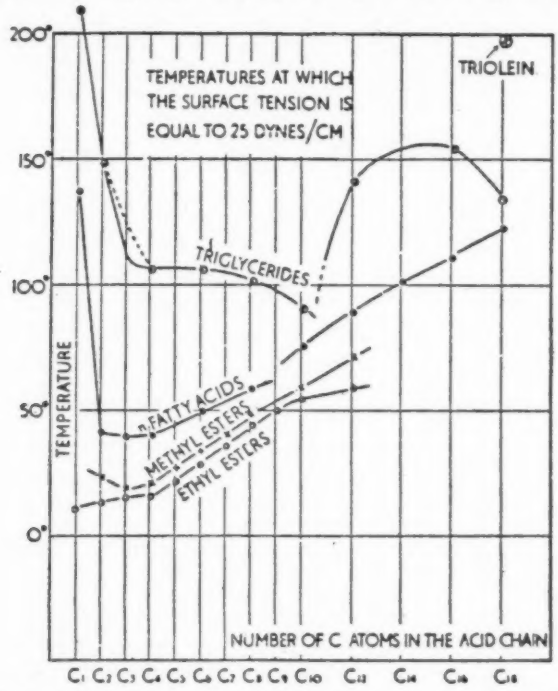

Fig. 6. The variation with chain length of the temperature at which the different members of a series have the same surface tension (25 dyne per cm).

strong molecular interactions; (2) C_3 to C_{10}, for which cohesion decreases slightly with increasing number of atoms; (3) above C_{14} with relatively higher values of surface tension but again with a tendency to decrease when the chain length increases. Between C_{10} and C_{14} (if not between C_{11} and C_{13}, for which data are lacking), there is a transition with rapid increase of the molecular interactions. In both curves (Figs. 5 and 6) the value for triolein stands apart and above the value for tristearin, as if, despite the lower melting point, cohesion in the unsaturated triglyceride was stronger than in the saturated one.

d. Ordinary fats

From measurements with various animal and vegetable oils by the drop method, CANALS and collaborators[9] have deduced that the surface tensions of these natural oils decrease linearly with the temperature. More precise determinations

between 20° and 130° have been done on the same type of oils more recently by HALPERN.[10] His data, relative to six different oils have been plotted in Fig. 7. One of the curves refers to a mineral oil (petrolatum) and is given for comparison. It should be noted that the temperature coefficient for the different oils (— 0·05

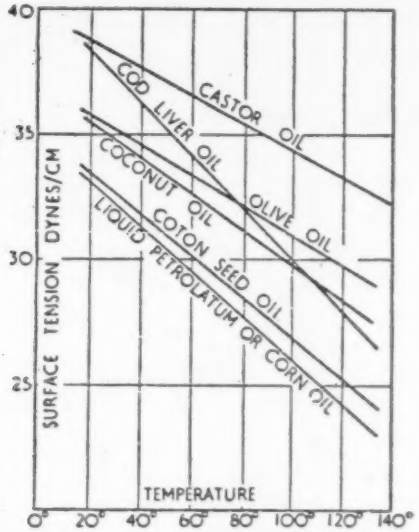

Fig. 7. Surface tensions at different temperatures of different ordinary fats.

to — 0·08) is comparable to those of the pure triglycerides. Cod-liver oil is a very particular case having a temperature coefficient of — 0·11.

2. *Semi-empirical general relations*

a. The parachor of fatty substances

If M, ρ, and γ are respectively the molecular weight, the density, and the surface tension of a substance, the quantity $P = (M/\rho)\gamma^{\frac{1}{4}}$ is independent of the temperature. This specific constant P was termed *parachor* by SUGDEN. What is important in SUGDEN's discovery is that P is an additive parameter, i.e. the parachor of a certain substance is equal to the sum of the individual parachors corresponding to the different groups forming the molecule, such as CH_3, CH_2, COOH, ester, etc. Definite corrections have to be introduced for double or triple bonds.

The parachors of the fatty acids have been determined in the work of HUNTEN and MAAS. They vary from $P = 169$ for propionic acid to $P = 778$ for stearic acid. Therefore the contribution of each CH_2 group to the parachor is *ca.* 40. From measurements on the series of long chain paraffins by VOGEL[11] the following values are deduced: $CH_2 = 40{\cdot}0$, $CH_3 = 55{\cdot}6$, C = 8·6, and H = 15·6.

The parachor of the different alkyl esters are given in the comprehensive study of VOGEL.[5] Both the methyl and the ethyl series of esters give an average value of 64 for the parachor of the —COO— ester group.

The mean parachor values calculated from JAEGER's data increase regularly in the series of saturated triglycerides from 700 for tributyrin to 2400 for

tristearin. Thus the mean increment for the whole molecule is 121 for every additional C atom in the acid chains. Therefore the contribution to the parachor of each CH_2 group in each of the three chains is again of the order of 40. For triolein, calculation of the parachor gives values oscillating from 2340 to 2370 between $-17°$ and $+90°$ and increasing afterwards up to 2420 at 185°. The maximum variation is therefore less than 3·5% despite the striking peculiarities of the surface tension-temperature curve (see Fig. 4).

b. The Eötvös constant of fatty substances

M/ρ being the molar volume, $(M/\rho)^{\frac{2}{3}}$ is equivalent to an area and represents a quantity proportional to the area per individual molecule on the surface. This gives the significance of the expression "molecular surface energy" used to designate the product $\gamma(M/\rho)^{\frac{2}{3}}$. That the surface energy will vanish when the critical temperature is reached ($T = T_c$) is obvious; but Eötvös's relation

$$\gamma(M/\rho)^{\frac{2}{3}} = K(T_c - T)$$

either under this original form or with an additive constant introduced by Ramsay and Shields, states furthermore that the molecular surface energy is a linear function of the temperature and that K is the same for all liquids. It is a striking fact that, with many hundreds of liquid substances investigated, the value of K remains between 2·0 and 2·5. For another group of substances, which contain an OH group in their molecule (amongst which is water), K varies between 1 and 2.

The interesting point here is that the most important deviations of this coefficient K are shown by long chain organic compounds and specially by fatty substances. With the triglycerides for example, K may reach values above 6 and varies greatly with the temperature. The values of K for the fatty acids, which have been calculated in the work of Hunten and Maas, are constant with temperature but they increase quite regularly from 0·9 for formic acid to 3·0 for stearic acid. Values of K for the different esters, which can be calculated from the data of Vogel, are also distributed along a line which rises, with the increase of the total number of carbon atoms, from *ca.* 2·1 for C_3 to *ca.* 3·2 for C_{16}.

To explain the high but constant values found for the long chain acids, Hunten and Maas suggested that they might be due to an orientation of the molecules on the surface. Rideal[12] has given a similar explanation. It is obvious that, while the *actual* area per molecule is independent of the chain length if the molecules are oriented (i.e. stand more or less erect) on the surface, the area *calculated* from the molecular volume $(M/\rho)^{\frac{2}{3}}$ can only increase with the number of carbon atoms. Assuming that the actual area per molecule is of the order of 25 Å^2 whatever the chain length, Dervichian[13] showed that the coefficient K for all the long chain acids and alcohols is reduced to a common value of *ca.* 1·1, which seems to be the value for all compounds with an OH group and which can associate through H bonding. On the other hand, a similar calculation reduces the value of K for the long chain esters and hydrocarbons to *ca.* 2, a value given by the great majority of substances.

3. *Molecular orientation at the surface of fatty substances*

There is no direct and unquestionable proof of the orientation of molecules at the surface of liquid and solid fatty substances, and arguments of the sort given at the end of the preceding paragraph are rather meagre.

The partly solidified surface of drops of triglycerides, fatty acids and paraffins have been submitted to X-ray diffraction by TRILLAT and NOWAKOWSKI[14] using a grazing beam tangent to the surface of the drop. The existence of a definite "orientation" has thus been proved in the very thin *solidified* skin which covers the drop of melted substance. What is meant here is that the crystallites formed on the surface have a common orientation, i.e. that the well-known long periodicity which appears in the crystalline structure of fats (see MALKIN, Vol. 1), and which corresponds to the "height" of two superposed elongated molecules, is directed perpendicularly to the surface of the drop. Nothing, however, can be deduced concerning the properly called orientation in the outermost layer of molecules, i.e. whether the polar groups of these molecules are turned outside or inside the liquid cannot be affirmed. In fact, the same type of organization was found by these authors in the solidified skin of paraffins, for which any consideration of inward or outward attraction on one end of the molecule is irrelevant. Strictly speaking, the results of TRILLAT and NOWAKOWSKI concern only the solid surface. Nevertheless, it is probable that at the surface of the liquid fats, in view of the discontinuity, the molecules have a certain roughly parallel and erect arrangement in which they are entrapped by solidification.

The structure of the surface of liquid normal fatty acids from C_1 to C_{10} was studied by BOUHET[15] by measuring the elliptical polarization of light after reflection on their surface. Using a theoretical relation due to DRUDE, the thickness of the limiting layer can be calculated from these measurements. The values of the thickness thus found remain between 5·5 and 6 Å, which is far from corresponding to the length of the molecules of the longer fatty acids. DRUDE'S theory contains, however, a few assumptions on the variation of the refractive index in the surface region which are far from being justified for the liquid fatty acids. All that can be concluded with certitude is that the transition from air to the inside of the liquid is gradual and not abrupt.

Observations which are in favour of the hypothesis that the hydrocarbon ends of the molecules emerge from the surface in contact with air will be mentioned later when the orientation at the contact with water will be considered.

4. *Adsorption and orientation of fatty substances at the surface of their solution in oils or organic solvents*

In a paper published in 1927, GILBERT[16] had shown that the surface tension of liquid petrolatum, which is 31·2 dyne per cm at 20°, is lowered to 30·4 by dissolving 0·0197 mol (i.e. 4·5 g) of myristic acid in 1000 g of it. The same lowering to 30·4 dyne per cm is obtained with 0·0517 mol (8·1 g) of pelargonic acid. A first point to be noted is that the surface tension of pelargonic acid at 20° is 29·5 dyne per cm. The greatest lowering to be hoped for by adding pelargonic

acid to the mineral oil would therefore be to the value of 29·5, when practically all the liquid would be constituted by the acid itself. The important fact is that the addition of only 8·1 g of pelargonic acid in 1000 of oil already brings the surface tension down to 30·4, while if an additivity effect was simply involved such a proportion would produce a lowering of less than 0·02 dyne per cm. Another point is that myristic acid is certainly much less soluble in hydrocarbons than is pelargonic acid and that less myristic acid is needed to produce the same effect. This relation between solubility and surface activity will be fully illustrated in the case of adsorption at the air-water interface.

More recently, DESNUELLE and MOLINES[17] have measured the surface tension of triglycerides in which less than 1% of phospholipids had been added. With one phospholipid, the addition of 0·6% lowered the surface tension from the original value of 36·6 to 33·5 dyne per cm. With another, it was lowered to 33·1 with an addition of 0·7%. Using an optical method due to MERIGOUX[18] these authors have been able to detect the presence, on top of the oil, of a film of phospholipid which flows back with a certain pressure after it is swept aside. This film is far from being monomolecular in thickness: in some cases its thickness can be estimated to 0·55μ. What happens actually in the present case is an accumulation of the phospholipid in the surface region, the concentration decreasing gradually from the surface towards the interior of the oil. When measuring the surface tension of oils containing phospholipids by the ring method, a persistent film remains attached to the ring[19] which must evidently be attributed to the presence of this layer.

The question remains open as to how the molecules of fatty acid or phospholipid are oriented in the adsorbed layer at the surface of their solution in oils. GILBERT assumes that the adsorbed fatty acid molecules have their polar groups turned outside, the chains being more soluble in the hydrocarbon oil. This is also the picture given by DESNUELLE and MOLINES concerning the phospholipid molecules at the surface of the triglyceride. This argument, which is intimated from what is admitted for monolayers spread on water, actually suffers from lack of any even indirect evidence.

A paper by JONES and SAUNDERS[20] illustrates an extreme case for solutions of fatty acids in organic solvents. Here the solvent, nitromethane, is strongly polar and, from this point of view its case is similar to that of aqueous solutions which will be analyzed later. For pure nitromethane $\gamma = 36{\cdot}4$ dyne per cm. Normal fatty acids from C_2 to C_8 have been studied. Increasing concentrations of each of these acids in nitromethane lowers the surface tension to values as low as 27·2 dyne per cm. The surface activity of the fatty acids increases with the chain length, e.g. if, with the C_7 acid, a molar fraction of only 0·15 is needed to obtain a surface tension of 29·4 dyne per cm, more than a molar fraction of 0·6 is needed with acetic acid to get the same lowering. From the extreme concentrations used by these authors, it appears clearly that they have been limited by the solubility which decreases with the chain length. The surface activity is therefore roughly inversely proportional to the solubility. This general rule will be amply illustrated in the case of aqueous solutions.

II. The Surface of Fatty Substances in Contact with Water

The existence in the molecules of fatty substances of a polar group (either acid or ester), with which water molecules can interact (mainly by dipole-dipole forces) more strongly than with the hydrocarbon groups, creates very particular conditions at the contact between water and these substances. Determination of the interfacial tension constitutes, even more here, an excellent indirect way to obtain information not only about the structure of the interface but also about the interactions across this surface.

1. *Interfacial tension between water and fatty substances*

The free energy of the interface between two liquids 1 and 2 (and consequently the interfacial tension $\gamma_{1.2}$) is related to the surface free energies γ_1 and γ_2 of each of the two liquids by the relation of Dupré:

$$\gamma_{1.2} = \gamma_1 + \gamma_2 - W_{1.2}$$

$W_{1.2}$ represents the *work of adhesion*, i.e. the work which must be spent at constant temperature in order to separate the two liquids in contact and which is somehow an estimate of the attraction between the two species of molecules.

The opinion that the interfacial tension $\gamma_{1.2}$ between an organic liquid and water depending on their mutual affinity (of which $W_{1.2}$ is a measure in the relation of Dupré) is therefore in close relation with the mutual solubility of the liquids has been more or less clearly expressed for a long time by different authors.[21], [3], [22] But the most convincing proof that $\gamma_{1.2}$ is a continuously decreasing function of the *mutual* solubility of water and a great variety of organic liquids has been given in a recent publication of Donahue and Bartell.[23] The interfacial tensions at various temperatures between water and different liquid substances, with which we are particularly concerned here, have been grouped in Table 1. These values have been taken from different publications which are indicated by superior reference numbers in parenthesis.

Table 1. Interfacial tensions against water

Acids and alcohols			*Esters and glycerides*		
	°C	dyne/cm		°C	dyne/cm
n-Butyl alcohol	25°	1·8[23]	Monostearin	70°	1·0[34]
Isovalerianic acid	20°	2·7[3]	Ethyl acetate	25°	6·8[23]
Caproic acid	20°	5·2[33]	Butyl acetate	25°	14·5[23]
Heptylic acid	20°	6·6[3]	Amyl acetate	70°	10·0[34]
Caprylic acid	18°	8·2[18]	Ethyl butyrate	30°	14·8[22]
Caprylic acid	60°	8·0[33]	Ethyl nonylate	20°	23·9[3]
Octyl alcohol	16°	8·7[24]	Ethyl caprinate	60°	22·0[33]
Undecylenic acid	25°	10·1[3]	Ethyl laurate	60°	25·0[33]
Lauric acid	60°	10·5[33]	Ethyl palmitate	60°	26·5[33]
Ricinoleic acid	16°	14·2[3]	Triolein	16°	20·4[34]
Oleic acid	20°	15·7[3]	Cotton seed oil	70°	28·0[34]
Oleic acid	60°	15·0[33]	Octane	25°	50·2[23]

From the examination of this table, it appears most evident that, in both the series of fatty acids and that of esters, the interfacial tension against water is related to the chain length or more directly to the solubility of the fatty substance in water. This latter point of view has the advantage of including also the action of temperature: e.g. amyl acetate, although containing more carbon atoms, has a lower interfacial tension at 70° than butyl acetate at 25°, but increase of temperature also enhances the mutual solubility. In the main, the interfacial tensions between water and the esters are higher than those between water and the fatty acids. This is certainly related to the lower affinity for water of the ester group in comparison to the affinity of the acid group. But, despite its COOH group, oleic acid in contact with water has an interfacial tension rather higher than that given by ethyl butyrate which is an ester. This shows that the solubility of the whole of the molecule has to be taken into consideration, a short chain ester being more soluble than a long chain acid. The fact that the deciding factor lies nevertheless in the nature of the polar group is shown when compounds of the same chain length are compared: e.g. there is no measurable interfacial tension between water and butyric acid, while ethyl butyrate gives 14·8 dyne per cm at 30°C.

With a given fatty acid the solubility can be increased, and hence the interfacial tension lowered, by ionizing the COOH group. Thus, by increasing the *p*H of the water in contact with a fatty acid, the interfacial tension decreases. As an extreme case, if a sufficient amount of alkali is introduced in the water, the interface may even vanish and the fatty acid may dissolve in the form of soap. The influence of *p*H on the interfacial tension between water and fatty substances was studied by HARTRIDGE and PETERS.[24] With oleic acid against distilled water at 16°, an interfacial tension of 12·7 dyne per cm was found, which incidentally is lower than the value found by other authors and is perhaps due to the impurity of the acid used. The important observation, however, is that the same value is found at different H ion concentrations below *p*H 5·6; the interfacial tension decreases with increasing *p*H reaching the value of 1·4 dyne per cm for *p*H 7·6. This change is also related to the concentration of salt in the water phase, for if, instead of using the normal quantity of buffer (0·05 *M* of phosphate) this is diluted to 1/10, the interfacial tension rises to 12·8 dyne per cm at *p*H 7·6. Similar effects were found with ricinoleic acid.

This change of the interfacial tension above a certain value of the *p*H is evidently related to the ionization of the acid group. It is more difficult to explain the fall of the interfacial tension between water and triglycerides or long chain esters when the *p*H is increased above 5, unless the presence of traces of free fatty acids may be suspected in these oils. This is highly probable in the case of the olive oil used by HARTRIDGE and PETERS, although it is claimed that it was purified by boiling with water; with this oil the interfacial tension was vanishingly small at *p*H 9·2. However, with pure triolein as well the interfacial tension at 16·3° fell from 20·4 to 12·1 dyne per cm when *p*H 7·4 was reached. These facts will be discussed later in the section on the water-solvent interface.

The specific action of the polar group appears much more clearly when the

adhesion work $W_{1,2}$, calculated from the Dupré relation, is considered instead of the interfacial tension. However, in order to compare the different type of molecules, the corresponding adhesion works should be calculated for the same

Table 2. Adhesion work (erg/cm²)

Fatty acids			*Esters and oils*		
	°C	erg/cm²		°C	erg/cm²
C_4 Butyric	20°	100	Ethyl acetate	20°	89·8
C_4 Butyric	60°	89	Ethyl acetate	60°	(80)
C_5 Isovaleric	20°	95·4	n-Butyl acetate	20°	83·5
C_6 Caproic	20°	95·4	Ethyl butyrate	20°	82·1
C_6 Caproic	60°	(86·8)	Ethyl butyrate	60°	(73)
C_7 Heptylic	20°	94·5	Ethyl caproate	20°	73·1
C_8 Octylic	20°	93·4	Ethyl nonylate	20°	76·6
C_8 Octylic	60°	83·0	Ethyl nonylate	60°	(68)
C_{18} Oleic	20°	89·6	Ethyl caprinate	60°	68·7
C_{18} Oleic	60°	(80·7)	Ethyl laurate	20°	74·6
C_{18} Ricinoleic	20°	94·2	Ethyl laurate	60°	66·1
			Ethyl palmitate	60°	65·2
			Triolein	20°	80·1
			Cotton seed oil	70°	68·4
			Octane	20°	44

temperature. This has been done for 20° and 60° using the surface tension data of Figs. 1, 3, and 4 and the interfacial tension values gathered in Table 1. Some of the latter had to be extrapolated for the temperature of 20° or 60°. For water $\gamma = 72{\cdot}8$ dyne per cm was taken for 20° and $\gamma = 66{\cdot}2$ for 60°. Table 2 gives the

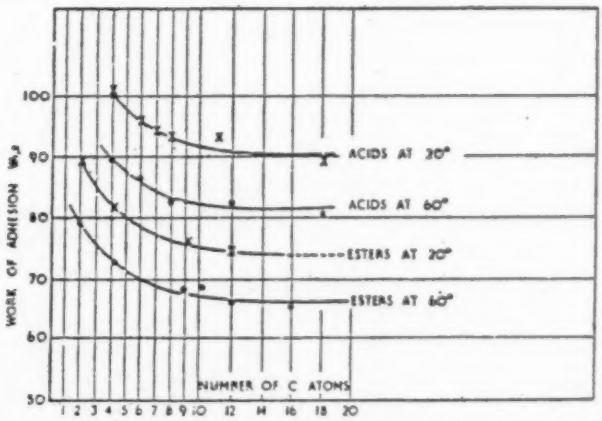

Fig. 8. The work of adhesion shown as a function of the chain length for the fatty acids and their esters at two different temperatures (20° and 60°).

calculated values of $W_{1,2}$ for different fatty acids, esters and oils. By plotting, as a function of the chain length, the values concerning the fatty acids and ethyl esters (Fig. 8), it appears clearly that, at a given temperature, the work of

adhesion to water does not vary much with the chain length of the molecule when the latter contains more than 8 or 10 C atoms. As would be expected, the work of adhesion at a given temperature decreases with the decrease of affinity of the functional group for water. The important point, however, is that above a certain chain length only the polar group of the molecule seems to be implicated in the adhesion to water. For the shorter chains the solubility of the entire molecule seems to intervene.

2. *Orientation of fatty substances at the contact with water*

HARDY[21] was the first to find that the work of adhesion to water is determined by the nature of the polar group present in the molecule of an organic liquid. He also was the first to deduce from this that on the interface the molecules of the organic liquid must be oriented with their polar groups in contact with the water.

Is there any direct proof of this orientation? The same x-ray method which they had utilized for the examination of the surface of fats was applied by TRILLAT and NOWAKOWSKI[14] to the study of very thin films of fatty substances solidified at the surface of a drop of water. There is undoubtedly a marked crystalline orientation in these films with the sheets of erect molecules parallel to the surface, but strictly speaking this result does not give any information about how the molecules are directed in the first layer in contact with the water.

DEVAUX[25] has tried to give an answer to this question. Fatty substances which have been melted on hot water give, after cooling, plates with faces having very different wettabilities. Water rolls on the upper face, while it adheres on the lower face forming either drops or a continuous film. Similar experiments were done by ADAM and JESSOP[26] with long chain acids and alcohols by measuring the contact angle with water. This angle was practically the same as with paraffin wax for the surface which was in contact with air during solidification. The experiments failed, however, when an attempt to study the surface solidified in contact with water was made. NIETZ[27] was more successful in measuring smaller contact angles with this particular surface. He was able to show an alternating variation between the odd and even fatty acids.

It should be emphasized that the contact angle between a solid surface and a liquid which has a strong affinity for it must not necessarily be zero. On the contrary, as a consequence of the affinity, the molecules anchor to the solid surface and prevent the liquid from advancing. However, once the surface is wetted, the liquid remains attached. This is the conclusion to which experiments of WOOG[28] on the spreading of different oils on the surface of metals lead.

III. ACTION OF FATTY SUBSTANCES ON THE WATER-ORGANIC SOLVENT INTERFACE

1. *Fatty substances dissolved in the organic liquid phase*

The interfacial tension between a paraffin oil and water is relatively large as a consequence of the weak mutual affinity of the two liquids; but if a fatty acid is

dissolved in the paraffin oil, it produces a decrease of the interfacial tension. The same phenomenon occurs if the fatty acid is dissolved in a triglyceride. These facts were first established by DONNAN[29] in 1899. Actually, a certain length of time is required after the water has been put in contact with the organic solvent, before an equilibrium value of the interfacial tension is reached. During this interval, which may last from a few minutes to several hours, the interfacial tension decreases more or less exponentially, the greatest fall occurring at the beginning. A recent analysis of the kinetics of this phenomenon, which is due to the progressive adsorption of the dissolved substance at the interface, may be found in three articles by WARD and TORDAI.[30] The method employed should therefore be such as to permit measurements at varying intervals after the formation of the interface.

Thanks to the systematic determinations of different authors, it has been possible to collect in Tables 3 and 4 the interfacial tension values against water

Table 3. Interfacial tensions against water ($\gamma_{1,2}$ in dynes/cm) of solutions in organic solvents

Acid	C_4	C_6		C_8					C_{10}	C_{12}		C_{14}	*Oleic*
Solvent	*Benzene*	*Benzene*	*Heptane*	*Nitrobenzene*	*Benzene*	*Cyclohexane*	*Tetradecane*	*Heptane*	*Benzene*	*Benzene*	*Heptane*	*Benzene*	*Heptane*
Mole/1000 g solv.	20°	23°	20°	25°	23°	25°	25°	60°	60°	23°	60°	25°	60°
0	34·6	34·5	50·8	24·9	34·5	48·2	48·4	50·8	34·5	34·5	50·0	35·1	50·0
0·02	30·6	32·5	36·5	24·0	32·5	37·0	35·5	38·5	33·2	34·2	39·1	34·2	40·25
0·05	28·5	30·4	33·0	23·0	29·5	33·6	31·5	35·0	31·2	32·7	36·0	33·0	37·3
0·1	26·4	28·2	29·5	21·5	27·5	31·0	28·8	32·3	29·2	30·0	33·5	31·8	35·0
0·2	23·6	26·0	26·4	20·0	25·7	27·9	26·0	29·9	26·8	26·3	31·1	30·7	32·7
0·5	20·4	22·5	22·0	17·2	23·3	24·0	21·6	24·0	23·8	23·75	27·1	28·5	29·5
1	17·3	19·0	18·3	15·0	21·4	21·0	18·5	21·1	20·0	22·30	23·5	25·8	26·65
2	13·7	17·5	15·4	13·5	19·0		15·4	18·1	18·3		20·4	23·5	24·0
10	5·1		9·2		(13·5)						14·2		18·3
30	3·3							9·8			12·3		16·5
Pure acid	(0)	(5·2)	(5·2)					9·8	(10)		(10·5)		(15·0)

of solutions of fatty acids and esters in different solvents at different temperatures and concentrations. In order to be easily comparable, the interfacial tensions have been interpolated from the data of the different authors for a series of chosen concentrations. The data for butyric acid have been taken from the old work of HARKINS and KING.[31] The data on other fatty acids dissolved in benzene, tetradecane, cyclohexane, and nitrobenzene are taken from two more recent papers by HUTCHINSON.[32] Interfacial tension values with heptane containing fatty acids or long chain ethyl esters have been measured by SEELICH.[33] These two last authors have measured equilibrium values.

Table 4. Interfacial tensions against water ($\gamma_{1,2}$ in dynes/cm) of solutions in organic solvents

Concentration in mole per 1000 g heptane	*Ethyl caprinate 60°*	*Ethyl laurate 60°*	*Ethyl palmitate 60°*
0	(50·0)	(50·0)	(50·0)
0·02	47·0	47·0	47·5
0·05	45·5	45·5	45·95
0·10	44·2	44·2	44·8
0·20	43·0	43·0	43·1
0·50	39·4	39·7	40·9
1	36·3	36·7	38·2
2	34·2	34·0	35·7
10	28·8	28·5	30·3
30	23·5	26·5	28·3
Pure ester	22·0	25·0	26·5

It appears from these tables that the value of the interfacial tension given by the solvent itself ceases to have any importance even for relatively small concentrations of the dissolved fatty substance. For example, although the interfacial tension of heptane against water is 50·8 and that of benzene only 34·5, solutions of the C_6 acid in heptane, above 0·2 mol per 1000 g, give lower interfacial tensions than the corresponding solutions in benzene. It appears also that values of the interfacial tension given by solutions of different acids in organic solvents increase with their length, as for the pure acids (e.g. $C_8 < C_{12} <$ oleic in heptane at 60°). For a given chain length, the addition of an acid to a solvent lowers the interfacial tension more than the addition of an ester. This is similar to the conclusion made in the preceding section concerning the water-fatty substance interface.

A still greater lowering of the interfacial tension is produced by the series of monoglycerides. Thus, GROS and FEUGE[34] have found that 0·7% (i.e. 0·02 mol in 1000 g) of monostearin at 70° lowers the interfacial tension of a mineral oil against water from 48 to only 1 dyne per cm. This important effect is evidently related to the greater solubility in water of the polar end of this molecule, which in addition to one ester group has two free OH groups. The same authors have shown that the lowering of the interfacial tension is also related to the length as well as to the nature of the hydrocarbon chain of the dissolved monoglyceride, when the solvent is a mineral oil.

The nature of the solvent has, nevertheless, to be taken into consideration. As noted above, benzene and heptane or tetradecane show minor differences in the interfacial tension at a given temperature when acting as solvent for equivalent quantities of a fatty acid; but caprylic acid, for example, gives much lower interfacial tensions against water when dissolved in nitrobenzene than when dissolved in cyclohexane. This might be in connection with the fact that fatty acids are generally less soluble in cyclohexane than in nitrobenzene

Change in *p*H of the water in contact with the organic solvent produces the

same variation in interfacial tension—and for the same reasons—as was described for the water/fatty acid interface. DONNAN had found that a paraffin oil containing 0·6% stearic or lauric acid showed a large decrease of the interfacial tension when in contact with water containing N/100 NaOH. He also observed the same effect with olive oil or rape oil containing free fatty acids. HARTRIDGE and PETERS[24], [35] examined the behaviour of solutions of fatty acids and long chain esters in benzene in contact with water at different *p*H values. The concentration of fatty substance was such that below *p*H 5 it produced little change of the interfacial tension, but with higher *p*H values a marked lowering occurred. It is difficult to understand the marked lowering of interfacial tension with esters and triglycerides at *p*H values of 8 or 9 when hydrolysis cannot yet occur to produce free fatty acids. Before offering any interpretation it should be confirmed that the esters contained no trace of free acid. It is, however, reasonable to admit, as was proposed by PETERS, that, even before the beginning of hydrolysis, less drastic interactions must occur between the ester group and the ions of water.

According to the thermodynamical relation of GIBBS, if the interfacial free energy between water and the organic solvent is lowered by the addition of a fatty substance, it follows that the latter must concentrate at the interface. Nearly all of the authors who have measured interfacial tensions have used the relation of GIBBS under different forms to calculate the excess fatty substance in the surface region and have found that it agrees fairly well with the assumption that it is under the form of an adsorbed single layer of molecules more or less oriented on the interface.

A very instructive experiment due to LANGMUIR[36] gives a more concrete demonstration of the mechanism of this adsorption. Pure paraffin oil does not spread on water, but if a large drop of paraffin oil containing stearic acid at a very weak concentration (10^{-5} to $5 \cdot 10^{-5}$ by weight) is deposited on the surface of acid water, it spreads slowly up to a certain limited area which is related to the amount of stearic acid contained in the drop. If now it is assumed that the spreading is due to the adsorption of stearic acid molecules on the interface, the proportionality of covered area to the quantity of fatty substance in the paraffin oil permits a calculation of how much stearic acid has come to the surface. This represents only a fraction of the total stearic acid showing that there is an equilibrium between what is on the surface and what remains inside the paraffin oil. If, on the contrary, the water is alkaline, then the drop expands much more and all the stearic acid comes to the surface and forms a monomolecular layer. This stronger adsorption on alkaline water is in complete agreement with the experiments of DONNAN or HARTRIDGE and PETERS.

It may be said that molecules of stearic acid coming in contact with alkaline water are entrapped since they become ionized and it is well known that soaps are much less soluble in organic solvents than are the corresponding fatty acids. On the other hand, at the contact with acid water they can be adsorbed and will redissolve according to a certain distribution equilibrium.

From what has already been said, it should be clear that lowering of the

interfacial tension with increasing *p*H is the consequence of two converging actions: (*a*) increase of the number of adsorbed molecules due to the decrease by ionization of their solubility in the organic solvent; (*b*) increase of the work of adhesion (affinity for water) as a consequence of the ionization of the COOH group.

2. *Fatty substances dissolved in the aqueous phase*

The question now to be considered is the behaviour of the interface between water and a liquid hydrocarbon when alkaline salts of the fatty acids are dissolved in the aqueous phase. Here again the classical work of DONNAN and POTTS[37] on the action of these salts on the interfacial tension between water and hydrocarbon oil appears to be of fundamental importance. Although the drop volume method was used without any correction and without allowing sufficient time for ageing of the interface, the results of DONNAN and POTTS retain their significance. Solutions at 17° of the sodium salts of 11 fatty acids, from C_2 to C_{14} at different concentrations were studied. Starting the series with the C_2 fatty acid salt, it was found that, for a given concentration (either $M/200$ or $M/400$) the interfacial tension began to fall significantly with the C_8 compound. From then on, the action increased rapidly with the chain length, so that with $M/200$ Na-myristate, the liquid flowed without forming drops. Much stronger concentrations were needed with the short chain acids to produce marked lowering of the interfacial tension.

Evidence of the adsorption of the soap at the interface can be found in the work of FISHER and HARKINS[38] who determined the amount of Na-oleate adsorbed on the surface of droplets in an emulsion. The adsorbed quantity was calculated by determining the change in concentration of soap in the aqueous phase. The area of the adsorbing surface was determined by measuring the mean diameter of the droplets into which a known volume of oil had been dispersed. The area per molecule adsorbed on the interface varied between 45 $Å^2$ and 20 $Å^2$ according to the age of the emulsion.

From what we know about the relation between ionization of the COOH groups in contact with water and the value of the interfacial tension, it is not surprising to find that increase in the *p*H of the soap solution in contact with a liquid hydrocarbon decreases the interfacial tension. Indeed this fact had been noted long ago both by ELLEDGE and ISHERWOOD[39] and by SHORTER[40] who found that the addition to the soap solution of various alkalies, such as NaOH, KOH, Na_2CO_3, or trisodium phosphate, increased the number of drops. On the other hand, if bi-carbonate is added to the soap solution, which originally had a *p*H of *ca.* 9·5, the *p*H will be lowered towards *p*H 8 and the interfacial tension will increase, as was observed by POWNEY and ADDISON.[41] These authors have studied systematically the variation of the interfacial tension between xylene and different soap solutions at different concentrations, and *p*H values. With all these variables, the complete picture appears very complicated, especially if the temperature is not taken into account.

The complications are mainly due to changes of state in the soap solution

itself. It happens, for example, that although at the same temperature (or rather because they are at the same temperature) and concentration, solutions of two different soaps are in completely different states. This is not the place to describe the constitution of soap solutions, but a few facts should be recalled in order to understand their surface properties. There is a temperature below which a soap crystallizes from its solutions. This temperature, called *Krafft point*, varies with the chain length of the fatty acid and with the nature of the cation. Thus at room temperature, the sodium soaps of all the acids from C_{12} to C_{18} (with the exception of the unsaturated compounds) are below their Krafft point and Na-myristate, for example, dissolves completely only above 55°. On the other hand, K-laurate, at room temperature, is already above its Krafft point.

In the complete miscibility region (i.e. above the corresponding Krafft point) there is a critical concentration for each soap, below which it is dissolved in the form of separate molecules. For higher concentrations all the rest of the soap is dissolved in the form of micelles constituted by the association of a number of molecules. It might be said that the critical concentration corresponds in a way to the saturation of the *molecular* solution and indeed many properties nearly reach a limit at this concentration. The important point is that the critical concentration decreases when the chain length of the fatty acid increases. It also decreases, for a given soap solution, when the *p*H is lowered or if increasing amounts of electrolytes are introduced. Generally speaking, any factor which decreases the solubility also decreases the critical concentration for micelle formation: i.e. it produces saturation of the molecular solution at lower concentrations.

The bearing of these different factors on the surface properties can now be considered. The passage from complete dispersion in water to precipitation under the form of crystallites when the temperature falls below the Krafft point, corresponds evidently to a sudden decrease in solubility of the soap molecules. The adsorbed layer will therefore produce a minor decrease in the interfacial tension. It is thus possible to understand the results of HIROSE and SHIMOMURA[42] who determined the interfacial tension, by the drop volume method, between petroleum oil and solutions of soaps of various saturated and unsaturated fatty acids at different concentrations. Below 40°, Na-stearate shows no significant effect, while Na-oleate, and even more linolenate and linolate, produce marked lowering of the interfacial tension. At 80°, the action of stearate is comparable with, if not higher than, that of oleate. It is sufficient to note that the Krafft point of Na-stearate is *ca.* 70° and that of the sodium salts of the unsaturated acids quite below room temperature.

If a constant alkaline *p*H is maintained, the interfacial tension decreases regularly with increasing concentration of soap in the aqueous phase until the critical concentration is reached and then remains practically constant, as if indeed the solution had been saturated. Thus the critical concentration is attained at much lower concentrations when the fatty acid chain is longer (e.g. 0·02 molar for Na-laurate and 0·0007 molar for Na-palmitate at 70° and in

presence of 0·1% of NaOH). It follows that a much smaller concentration of a fatty acid salt will produce a given decrease in interfacial tension when the chain is long than when it is short (see POWNEY and ADDISON[43] for example). Therefore, if comparative measurements are made with soaps of fatty acids of different chain length at the same molar concentration and at room temperature, as was done by CAVIER,[44] it will appear that the interfacial tension decreases very rapidly with the chain length up to about C_{14}. However, as the temperature is below the Krafft points of the longer chains, the solutions of palmitate and stearate will seem less active. The resulting effect, due to these two opposing factors, will be that of a maximum action with the C_{12} or C_{14} acid; yet the unsaturated C_{18} acid salts will show a markedly strong action. This maximum with the C_{12} or C_{14} chain will obviously disappear if the comparative study is made at a temperature (e.g. 80°) at which even the stearate is above its Krafft point. Quite often the determinations reported in the literature concern Na soap solutions which have been studied at room temperature (i.e. below their Krafft point). This adds unnecessary complications arising from the metastable and therefore hardly reproducible state of a soap solution when it is cooled to a temperature at which it eventually precipitates.

It is easy to understand that, as the addition of an electrolyte decreases the critical concentration, the addition of, for example, NaCl to a soap solution will be equivalent in certain respects to increasing its concentration. The result will be a lowering of the interfacial tension with increasing quantities of NaCl or KCl as observed by SHORTER[40] and by POWNEY and ADDISON.[41] These last workers found that the addition of increasing quantities of a mixture of Na_2CO_3 + $NaHCO_3$ produces first an increase of $\gamma_{1,2}$ and then a decrease.

Fatty acids being weak acids, their salts undergo hydrolysis with dilution and when the *p*H is lowered. From this, new complications arise. One of the consequences, namely the change in critical concentration, has already been mentioned. Another, which has been considered by POWNEY and ADDISON[41] and by DAVIS and BARTELL,[45] is the diffusion of the liberated free acid through the interface into the organic phase where it is soluble. Thus, the length of time necessary for the interfacial tension to reach a steady value is increased considerably by the time required for diffusion of the free acid to reach equilibrium.

It has already been mentioned how, in relation to *p*H, the degree of ionization of the adsorbed molecules affects the work of adhesion and consequently the interfacial tension. Moreover, for each concentration of soap and for each chain length of fatty acid, a different extent of hydrolysis occurs at different *p*H values. This in fact changes the composition of the solute and therefore its solubility (i.e. its critical concentration). The variety of reported results obtained by changing both *p*H and concentration can thus be understood. It is significant that, by adding a sufficient amount of NaOH (0·1%) to suppress hydrolysis at any concentration and by doing measurements at 70°, POWNEY and ADDISON[41] have obtained much more coherent results with varying chain lengths of the fatty acid.

3. *Phosphatides dissolved in triglycerides*

The interfacial tension between triglycerides and water is modified by the presence of phosphatides in the oil phase. Here again the equilibrium value is reached sometimes only after 30 min. The lowering of $\gamma_{1.2}$ is a function of the concentration of phosphatide. With a certain sample of triglycerides, investigated by DESNUELLE, MOLINES, and DERVICHIAN,[46] $\gamma_{1.2}$ fell from the original value of 30 dyne per cm to only 8 dyne per cm by adding 0·11% phosphatide dissolved in the oil phase. Phosphatides which are soluble in the oil phase do not dissolve, but only swell, in water; their affinity for water is therefore greater than that of fatty acids. This character explains the considerable lowering produced in $\gamma_{1.2}$ and also the fact that their adsorption at the water-oil interface is not merely limited to the formation of a single layer. After a few hours of contact, a visible skin is formed at the interface which is itself a phase swollen by water. Details about the nature of this phase, composed of triglycerides, phosphatides and water associated in certain proportions, can be found in the original paper.

Visible films appearing at the interface between different oils (olive, castor, cod-liver, mineral) and aqueous solutions of different emulsifying agents (Na-oleate, glycocholate, gelatin, saponin) after a contact of 24 hr to 15 days have been described by SERRALACH and JONES.[47] They are probably similar to those obtained with phosphatides and described above.

4. *The direct spreading of substances at the water-oil interface*

Instead of leaving a soluble substance to be adsorbed at the interface from its solution either in the water or the organic phase, substances, which are practically insoluble in both of the liquids, can be brought into contact directly with the interface on which they immediately expand.

If the molecules of the substance are of the polar–non-polar type, orientation on the interface places each of their two parts in its most suitable solvent. While the substance cannot migrate in any other direction because of the insolubility of the whole of the molecule in both of the liquids, diffusion can occur with the greatest ease *in the plane* of the interface since each of the two ends of the molecule is maintained in its own solvent and moves in it. To this surface diffusion corresponds an interfacial osmotic pressure or simply *interfacial pressure* π. This pressure, which tends to expand the adsorbed layer of molecules, acts in opposition to the interfacial tension, which on the contrary tends to contract the interface. It can be demonstrated that, if $\gamma_{1.2}$ is the interfacial tension before the adsorption of the substance, the resulting value of the interfacial tension after spreading has occurred is:

$$\gamma_{1.2}' = \gamma_{1.2} - \pi$$

Inversely, the interfacial pressure of the adsorbed layer can be determined by measuring the lowering of the interfacial tension:

$$\pi = \gamma_{1.2} - \gamma_{1.2}'$$

In fact, ASKEW and DANIELLI[48] have measured directly the expansion pressure of substances spread at the interface between water and bromobenzene by adapting LANGMUIR's method (see next section on monolayers) to the study of this interface. Unfortunately, practically all fatty substances are more or less soluble in the organic phase, if not in the water, and they tend to leave the interface. Only a very long chain amide and an amino-acid could give interfacial films. ALEXANDER and TEORELL[49] studied the expansion of phosphatides at the water-benzene interface. They found that the addition of calcium ions to the water increases the anchorage to the interface of kephalin molecules which have evidently a tendency to diffuse into the benzene.

Despite its very great interest, this field of research is not yet developed.

IV. MONOLAYERS OF INSOLUBLE FATTY SUBSTANCES SPREAD AT THE SURFACE OF WATER

1. *Introduction and methods*

If the surface of water is touched with a platinum wire carrying a very small quantity of oleic acid or triolein, the fatty substance spreads very rapidly and remains on the surface. The phenomenon can be followed easily if the surface of the water is previously sprinkled with talcum powder: the spreading substance sweeps the surface pushing the grains of talcum to the edges of the trough which contains the water. The polar groups of the spreading molecules dissolve and diffuse on the surface, but the insolubility of the long hydrocarbon chain prevents any diffusion inwards. To this surface solution and diffusion corresponds a *surface pressure* π which can be defined similarly to the interfacial pressure at the end of the preceding section.

The surface tension of water is lowered from its original value γ to a new value γ' such that $\gamma' = \gamma - \pi$. This lowering of the surface tension of water by contamination led RAYLEIGH to the determination of the smallest amount of oil which could produce it and to the conclusion that it corresponded to the thickness of only one molecule. In fact there is no limit to the expansion of the fatty substance except the boundaries of the surface of the water, and the surface pressure decreases indefinitely with the indefinite expansion. If the area is large enough in comparison to the deposited substance, this will spread more and more until all the molecules come into contact with the surface of water, so that the thickness of the film is reduced to only one layer of molecules. Even then expansion proceeds and the molecules, while held on the surface, scatter away from each other as long as there is space available.

The way of sweeping the surface of water and of using barriers in order to vary the area, which are at the basis of the study of monomolecular films, is entirely due to AGNES POCKELS. Suppose that in front of the expanding film, a strip of thin paraffined mica foil is placed so as to float on the water and to bar all the width of the surface. Thus a clean space is isolated behind this float and, the invading film, under whose pressure the floating barrier can be carried away, is in front. Different mechanical devices can be imagined which enable one to

measure the force necessary to maintain the barrier in its zero position. In addition, a second barrier can be placed behind the film so as to border it at the opposite side to that of the first floating barrier. By moving this second barrier, the area available for the film can be varied. It will actually act as a "piston" compressing or expanding the surface film, while the first floating strip acts as the membrane of a manometer. This is the principle of the apparatus first imagined by LANGMUIR and to which different modifications have been brought by different authors. Technical details can be found in ADAM'S well known book.[50]

Consider a film of oleic acid or triolein which has been left to expand to a large extent and is then gradually compressed. The surface pressure increases with decreasing area until a point is reached where, through lack of space, the molecules leave the surface to collect into droplets. The film is said to *collapse*. It may also be said that there is passage from a two-dimensional state to a three-dimensional (ordinary) state. With a solid substance, such as stearic acid deposited on water at room temperature, spreading from the crystal is slow and stops when a relatively low pressure is reached. An important fact is that the spread monolayer can then be compressed to much higher pressures before it collapses. With increase of the temperature the spreading pressure also rises gradually until the melting point is reached, where the behaviour is similar to that of oleic acid. The surface pressure of spreading for different temperatures and different fatty acids and esters have been determined by CARY and RIDEAL.[51]

To facilitate the spreading of a solid substance it is dissolved in a volatile solvent, such as benzene or petroleum ether, which itself spreads readily on water. After evaporation of the solvent, the molecules of the substance remain spread on the surface. This method, due to DEVAUX, is used as well for liquid fatty substances, for it allows the determination of the quantity spread by preparing titrated solutions and measuring the deposited volume. Thus for every position of the piston, which fixes a certain surface on the LANGMUIR trough, the available area per milligram of substance or, following LANGMUIR, the area, expressed in square Ångström ($Å^2$), per individual molecule, can be calculated. With the corresponding measured values of the surface pressure for a given temperature, curves can be plotted which represent the surface pressure π as a function of the area per molecule A. These π — A isotherms are the two-dimensional analogues of the more familiar tri-dimensional pressure-volume (P — V) isotherms.

The contact potential between two phases corresponds to the work which is spent when electrons are carried from one phase into the other across the limiting surface. In one of the methods for measuring this potential at the water-air interface, an electrode is held a short distance above the surface of the water and the air gap between the two is ionized to make it conducting. An electrometer is placed between this electrode and another one which dips in the water. A zero method may be adopted by introducing a potentiometer and determining the electromotive force of the whole circuit by opposition. Details of construction

have been given by different authors and a recording apparatus has been described by DERVICHIAN.[52] The introduction of a dense monolayer on the surface of the water produces a change in the air-water contact potential which is of the order of many tenths of a volt. This shift of the potential is called the *surface potential* of the monolayer. It varies with the substance and with the area per molecule as well as with the *p*H and the salt concentration of the underlying aqueous phase.

The increase of surface viscosity of water due to the presence of a fluid monolayer can be determined by measuring the rate of flow of the monolayer through a long narrow channel under a constant difference of pressure at the two ends. This type of instrument was first introduced by DERVICHIAN and JOLY.[53] Another type of surface viscometer has been described more recently by CHAMINADE, DERVICHIAN, and JOLY[54] and is a two-dimensional adaptation of the Couette ordinary viscometer. Here a floating ring is rotated at a constant speed and the resisting force due to the presence of a monolayer is measured. The film is maintained under a given pressure in the gap between the rotating ring and an external floating but fixed flat ring.

The complete theory of the flowing of surface films has been developed by JOLY[55] and it is possible to calculate the viscosity proper to the monolayer itself from the measured values. In the case of the rotation method, the viscosity of the layer can also be calculated by assuming that the resisting torque due to the water alone and that due to the film itself are additive. Viscosities calculated from values measured with both methods coincide.

2. *Changes of state in monolayers*

A simple qualitative examination, by blowing on the surface after it has been sprinkled with talcum powder, enables one to distinguish a rigid or *solid* monolayer from a fluid or gelified one; but, strictly speaking, it is not possible to discriminate actually in this way and even by viscosity measurements the different types of fluid films (gaseous, liquid, or intermediate) nor the solid types. The differences can be rigorously established only by the comparative examination of the different isotherms.

a. Different types of isotherms and first indication of phase changes

As far as long chain fatty substances are concerned, experience shows that the areas which are characteristic of the different states are practically identical when expressed as area *per chain*: e.g. the molecular area at the triple point is *ca.* 38–39 Å^2 for the fatty acids and *ca.* 115–120 Å^2 (i.e. three times 38–39) for triglycerides. This enables one to describe the isotherms in a more general way and to define regions expressed in area per chain where each phase can exist.

The different types of isotherms given by monolayers of fatty substance can be summarized in the eight cases represented in Figs. 9 to 16. Each case refers to examples established by different authors on different members of the series of fatty acids, the esters or the glycerides.

Fig. 9 illustrates the extreme case where no discontinuity appears when the

monolayer is compressed from very large molecular areas and very weak surface pressures to areas at which the intermolecular distances are as small as the molecular dimensions themselves and the surface pressure reaches the order of a few tens of dynes per centimetre. A clear example of this case is given by

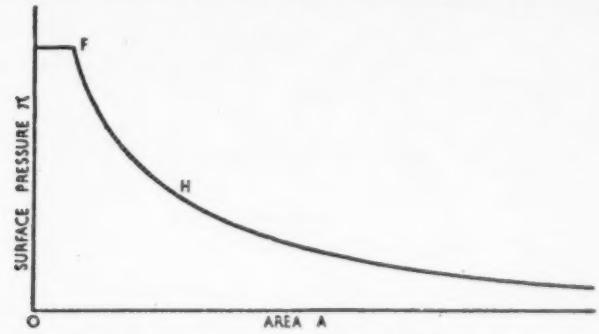

Fig. 9. A type of surface pressure—area isotherm which presents no discontinuity before the collapse point F (monolayer remaining gaseous up to the highest pressures).

tricaproin as shown by DERVICHIAN.[58] By analogy with the well-known P—V isotherms of ordinary gases, it might be said that the substance spread as a monolayer behaves as if it were above its critical temperature of liquefaction. In this case the monolayer is therefore comparable to a gas which can be compressed under high pressures to a relatively small volume without showing any

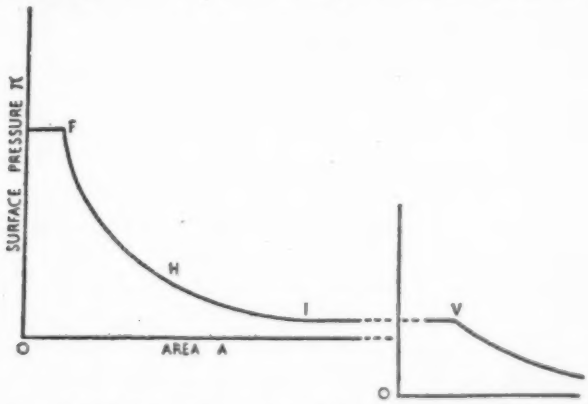

Fig. 10. A type of surface pressure—area isotherm showing a gas—liquid transition (V—I). In order to give a picture of the complete isotherm, pressures on the right-hand side of the diagram are represented on a much larger scale than on the left-hand side; areas, on the contrary, are on a smaller scale. With the scale of pressures used on the left-hand side, the horizontal part of the isotherm ending at point I would not actually be distinguished from the area axis. It has been purposely drawn quite above it.

phase change. However, the film collapses at the point F where the molecules leave the surface.

In the case illustrated by Fig. 10, a transition appears on the isotherm between a phase with a molecular area corresponding to the point V and another fluid phase I having a molecular area larger than 39 Å^2 per hydrocarbon chain. As

was shown by ADAM and JESSOP[56] for areas larger than V (e.g. 850 Å² for myristic acid, or 2400 Å² for C_{15} acid) the product πA of the surface pressure by the area (expressed in square centimetres per gram-molecule) tends with increasing areas towards the value of RT, where R is the gas constant. Indeed in such states of great expansion, the intermolecular distances in the monolayer are of the same order as the distances in an ordinary gas under relatively low pressure. By simple analogy monolayers in this state are called *gaseous*. Similarly, all along the transition from V to I, the gaseous phase, which can now be called a surface *vapour*, with a characteristic molecular area corresponding to the point V, is gradually transformed into a condensed but fluid state I in which

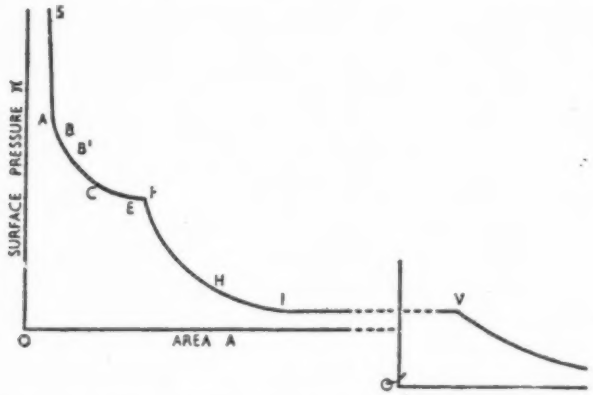

Fig. 11. A type of surface pressure—area isotherm showing, in addition to the gas—liquid transition (V—I), a second transition (F—E).

the area per chain has a well defined area corresponding to the point I and not inferior to 39 Å².

When compression proceeds, no other ordinary transition occurs and the surface pressure increases from I to F where the monolayer collapses. The area per chain at the point F varies between 27–29 Å² and the layer is still fluid. To this type belong the isotherms obtained by ADAM and JESSOP[57] with myristic acid at 34°, by DERVICHIAN[59] with triolein at room temperature and with trimyristin at 44°, or by HARKINS, YOUNG, and BOYD[60] with pentadecylic acid above 35°. It should be noted that the areas indicated by ADAM and JESSOP are consistently too large by about 10%.

In the case of Fig. 11, a transformation appears at F which leads finally to a solid state when the point A is reached. The film is very poorly compressible above A and it collapses at S. For a given substance on a given underlying aqueous solution, the higher the temperature, the higher the pressure, and consequently the smaller the area, at which the transformation at F occurs. Finally, above a certain temperature the isotherm is completely changed into the preceding form of Fig. 10. The area per chain at which this second transformation F commences thus remains between 39 Å² (lower temperature) and 27 Å² (higher temperature), while the area at the point A is equal to *ca.* 19·5 Å²:

e.g. myristic acid at 12°[57] and 21°,[59] trilaurin at 14°,[59] pentadecylic acid at 20°.[60]

Other monolayers, although giving the same kind of change which leads from F to A, do not show the transition from vapour V to fluid I, as if the passage from the gaseous phase to the state represented by F happened without dis-

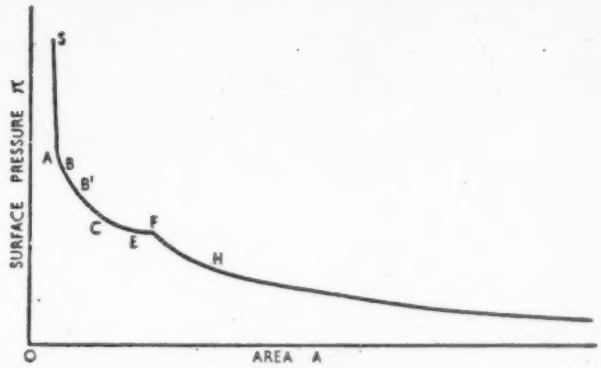

Fig. 12. A type of isotherm which does not present a gas-liquid transition (V—I) but which shows the transition F—E.

continuity. This is the case which is represented on Fig. 12 and which has been obtained by ADAM[61] with ethyl palmitate at 15°.

It was said above that by increasing the temperature the isotherm obtained with a substance may change from the form shown on Fig. 11 to the form represented on Fig. 10. By decreasing the temperature, the same substance may

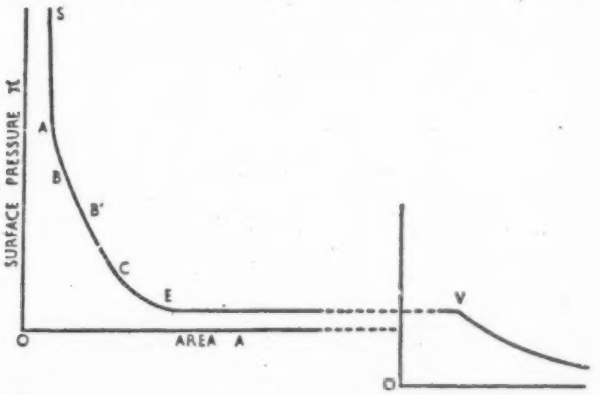

Fig. 13. A type of isotherm presenting a transition (V—E) from a vapour phase to a more condensed phase.

on the contrary, give an isotherm of the type represented on Fig. 13. This form of isotherm can also be obtained at room temperature with a homologous substance having a longer hydrocarbon chain. In this case, the condensation of the vapour phase starting at the point V leads to a new state E which, although still fluid, is more condensed than the state represented by the point I on Figs. 10 and 11. In fact the area per chain at the point E is lower than 33 Å^2. Examples

of this form of isotherm have been obtained with myristic acid at 4° by ADAM and JESSOP,[57] with palmitic acid at 25° by DERVICHIAN and JOLY[53] and by HARKINS, YOUNG, and BOYD,[60] or with trimyristin at 26° by DERVICHIAN.[59] Here, the condensed fluid layer which is less compressible than in the preceding

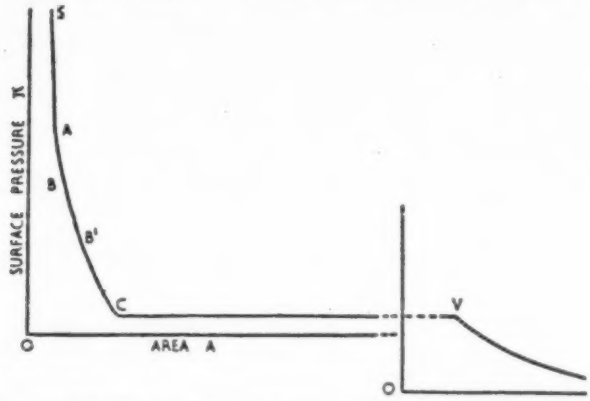

Fig. 14. A type of isotherm presenting a transition (V—C) from a vapour phase to a phase still more condensed than in the case of the preceding figure.

cases, solidifies at the point A and collapses at S. The area per chain at the point E may decrease below 33 Å^2, when the temperature is lowered or when compounds with a longer chain are utilized, up to a value of 24·5 Å^2. Then suddenly another type of isotherm can be obtained (Fig. 14) where the condensed fluid is still less compressible and has an area of 23·5 Å^2 at the point C.

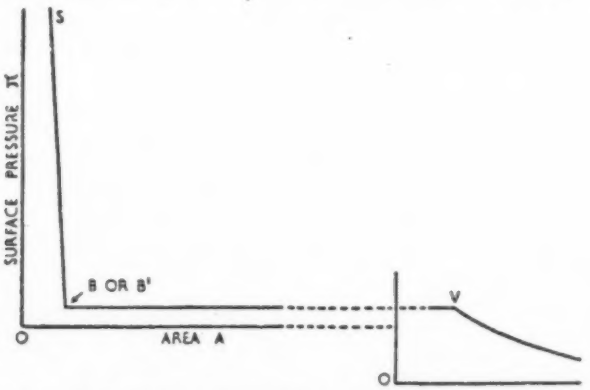

Fig. 15. A type of surface pressure—area isotherm presenting a transition V—B or V—B' of a vapour phase to a phase which is generally solid.

To this type belong the isotherms of stearic acid spread at 20° on N/1000 HCl or trimyristin at 22° given by DERVICHIAN[59] or that of the C_{20} acid spread at 25° on HCl N/100 given by STENHAGEN.[62]

At still lower temperatures or with compounds having many more carbon atoms in their molecules, the vapour phase at V is transformed directly into one of the solid phases whose area per chain is represented by one of the points B or

B' above which the compressibility of the layer is very small (Fig. 15). With different conditions (pH and temperature) the area at the point B is equal to 20·5 or 22 Å^2 per chain. This type of monolayer is given for example by trimyristin below 20°.[59] In some cases, the monolayer at the point B, although very condensed is still fluid. Furthermore, with compounds having more than 18 carbon atoms in their chain, transitions of the type indicated by $B'B''$ and BA' on

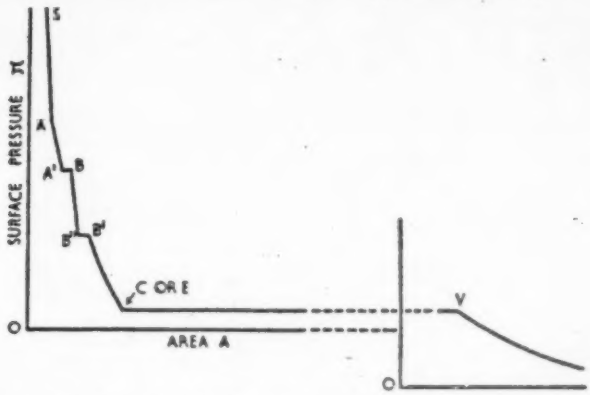

Fig. 16. A type of isotherm showing discontinuous transitions (B'—B'' and B—A') of the condensed phase itself in addition to the vapour-condensed phase transition (V—C).

Fig. 16 may appear in this highly condensed but fluid state. Such transitions (Fig. 16) have been observed by STENHAGEN[62] with the C_{22} acid at 10°.

b. Character and definition of different phases

The easiest way of defining the different states is to start from the most condensed state and study the change of the molecular area with increasing temperature. The discontinuities in the area which are thus found correspond to the formation of the successive states. This study has to be made, not under an arbitrary constant pressure, but under saturated vapour pressure; i.e. according to the Phase Rule, under conditions of one degree of freedom. In fact, it consists in determining, at different temperatures, the area of the condensed phase which remains in equilibrium with the vapour phase V, as represented on the different isotherms. Starting from the area of the point B or B' of Fig. 15, and gradually increasing the temperature, areas corresponding to the point C of Fig. 14, E of Fig. 13, I of Fig. 11 will be found successively at definite temperatures, which are considered as transition or melting points.

This variation of the area per chain, under vapour pressure, is represented on Fig. 17 for the series of triglycerides and on Fig. 18 for the series of the saturated fatty acids. The same peculiarities appear on both series of curves but in a different range of temperature for each compound. These temperature ranges are placed in the same order as the ordinary melting points of the considered series of substances. The first portion of the curve relative to the C_{22} acid has been established by using data derived from the series of isotherms given by STENHAGEN.[62] The rest of the curve (in dots) is simply hypothetical.

It is now possible to generalize. For each substance, there is a temperature T_l below which the monolayer, under vapour pressure, is in a very condensed state which is generally rigid and occupies an area per chain of 20·5 (B) or 22 Å² (B'). At this temperature, the area per chain increases suddenly to 23·5 Å² corresponding to a new phase (C) which is fluid and exists in a more or less wide interval extending up to another temperature T_m at which a continuous transformation commences. In this new fluid phase (E), the area per chain increases gradually with increasing temperature till an upper limit of 32–33 Å² per chain is reached.

Then a discontinuity appears at a definite temperature T_l when the area increases suddenly to the value of 38–39 Å² per chain. From this point on, the

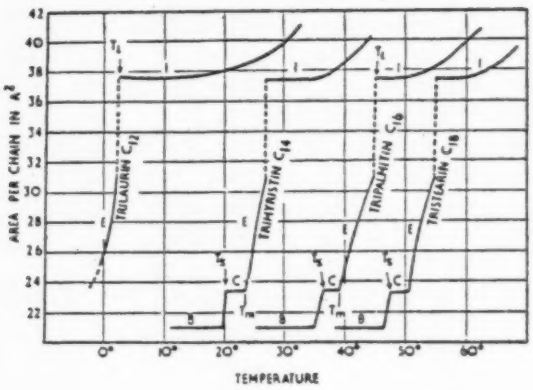

Fig. 17. Variation with temperature of the molecular area of the condensed phase in equilibrium with the vapour phase, for a series of triglycerides. The letters B, C, E, I correspond to the lettering used in the previous figures at the end of the vapour-condensed phase transition.

monolayer is in a new fluid state (I) which expands much more slowly with increasing temperature. Thus between T_m and T_l, which corresponds to roughly ten degrees, an expansion of the area of more than 60% occurs, first in a continuous way then suddenly. Just before T_l, the phase (E) in equilibrium with the vapour phase is characterized by an area of 32–33 Å²; just above this temperature, it is replaced by a new phase (I) with a characteristic area of 38–39 Å². Three phases can therefore exist in equilibrium at the temperature T_l which is a triple point.

Fig. 18 shows clearly that the same state C which occupies an area of 23·4–24 Å² per chain is found in a different range of temperature for each fatty acid. If therefore the different fatty acids were considered at the same temperature of 25°, on HCl N/100 for example, they would be in different states and the molecular areas would differ as can be seen on the figure: i.e. 24 Å for the C_{20} acid, 24·5 for the C_{18} acid and 32 Å² for the C_{16} acid. This is how Harkins and Boyd[63] have been misled when arguing that the molecular areas were not independent of the chain length.

It should be emphasized that the range of temperature in which each state can exist varies not only with the nature and chain length of the fatty substance, which seems quite evident, but also (specially with the fatty acids) with the pH and the electrolyte concentration of the underlying water. It is also self-evident

that the temperature at which changes occur in monolayers have nothing in common with the ordinary melting points of the spread substances, except that they are placed in the same relative order. Attention was drawn by DERVICHIAN[58] to the fact that the characteristic areas of 18·5, 19·5, 20·5, 22, 23·5 Å², which are found in different states in the monolayers, corresponded to identical areas per chain found in the three dimensional lattice of crystals of fatty substances. The characteristic area of 32–33 Å² has recently been compared by the author[64] to the area per chain in the para-crystalline "neat" phase given by anhydrous soaps. This correspondence does not necessarily mean an identity

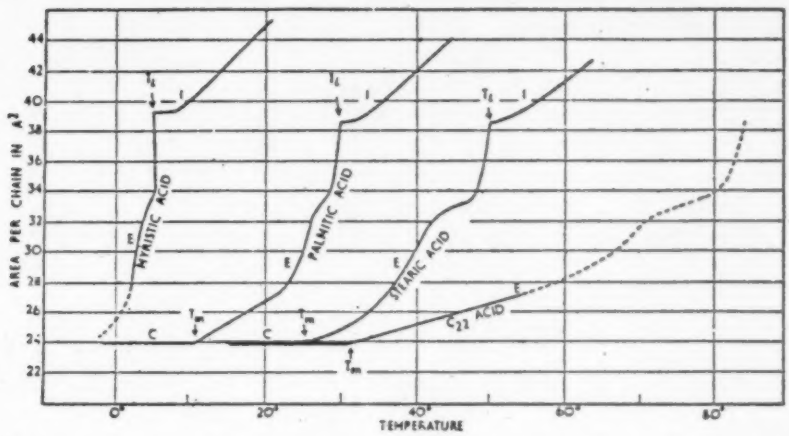

Fig. 18. Variation with temperature of the molecular area of the condensed phase in equilibrium with the vapour phase, for a series of fatty acids. The letters *B, C, E, I* correspond to the lettering used on the isotherms of the previous figures at the end of the vapour-condensed phase transition.

between two and three dimensional states, so much the less since some of the considered phases in monolayers are fluid. However, it seems natural to compare the mean intermolecular distance in the phase which is the last to appear when starting from the solid state (i.e. phase *I under vapour pressure*) to the mean distance between molecules in the ordinary liquid state of the same substances. From the comparison made in the above-mentioned paper[58] it appears that the distances are of the same order of magnitude and that the molecules in the ordinary liquid in three dimensions are certainly not more packed than in the surface phase at the point *I*. By simple analogy the term of *liquid* has therefore been applied by DERVICHIAN to this most expanded fluid surface state which appears in equilibrium with a surface gaseous phase.

The term *mesomorphous* has also been proposed by DERVICHIAN to designate the fluid but much more condensed phase which appears immediately after the solid at *C* on Figs. 17 and 18. This term means simply "intermediate form" and, although it was previously introduced to designate the well-known liquid crystal states, it does not necessarily imply that the state in the monolayer is identical to the state in a liquid crystal. Nevertheless, the packing of the molecules in this fluid surface phase is comparable to that in certain solid fatty acid

crystals. Its behaviour during thermal expansion is also strikingly comparable to that of the three-dimensional mesomorphous phases. Indeed, there is no discontinuous change of this phase at higher temperatures, but a gradual, although extraordinarily rapid, expansion above T_m under the form of a new phase E (see Figs. 17 and 18). The name of *expanded mesomorphous* has been given for this new phase which can expand from an area of 24 to 33 Å² per chain. The increase, under vapour pressure, from this area to the value of 38–39 Å² corresponding to the properly called *liquid* phase I, occurs, at a definite temperature T_l, only through a discontinuous transition.

The correspondence between the different proposed nomenclatures is given in Table 5.

Table 5

Adam[50]	Dervichian[59] (1937)	Harkins *and col.*[60] (1940)	Dervichian[64] (1952)
—	—	—	*Condensed solid**
Condensed: close packed chains	Solid	Solid	*Solid*
	—	Superliquid (LS)†	*Superliquid*
Condensed: close packed heads	Mesomorphous	Liquid 1 (L_1)	*Mesomorphous*
	Expanded mesomorphous	Intermediate (I)	*Expanded mesomorphous*
Liquid expanded . .	Liquid	Liquid 2 (L_2)	*Liquid*
Vapour expanded . .	Vapour or	Vapour or	*Vapour* or
Gaseous	Gaseous	Gaseous	*Gaseous*

* After Stenhagen.[43] A very condensed solid phase whose area per chain can be reduced up to 18·6 Å².
† A term introduced by Harkins and Copeland[47] to designate a fluid phase which appears under high pressure such as on Fig. 16 ($B'B''$ or BA').

Overlooking the fundamental distinction between the different fluid phases and the successive temperatures of their appearance, may lead to complete misunderstanding. Thus Alexander[65] missed the distinction between the mesomorphous phase and the proper *liquid* phase by asserting that cetyl alcohol was already liquid at room temperature and could not therefore have a melting point at 50°. Actually this temperature corresponds, for cetyl alcohol, to the point T_l (see Figs. 17 and 18) at which the real liquid phase appears, while the state at room temperature corresponds to the condensed, *mesomorphous*, phase (Fig. 14).

c. Phase transformation by pressure variation

Each of the phases which have been defined can be compressed under increased pressures along an isotherm, i.e. at constant temperature. The area can thus decrease and yet the phase remain the same. This is what happens in Fig. 11 when the area of the liquid phase is decreased from I to F; but at this point F, a transition to a new phase occurs. By a simple argument of continuity, it can be inferred that the new phase E which is formed is the expanded mesomorphous, since, by decreasing the temperature, the point F moves downwards on the isotherm and, at the lowest limit, the triple point can be reached where the

third phase in equilibrium with the vapour and the liquid is indeed the expanded mesomorphous. It can also be seen on Fig. 11 that, from the point F upwards, the isotherm is the replica of what it is above E on Fig. 13. That a transition occurs after the point F, is shown by the fact that the surface potential remains constant in this region between F and E. This was demonstrated by SCHULMAN and RIDEAL[66] and appears very clearly on recorded curves of the surface potential.[58]

As shown on Fig. 16, with mesomorphous layers of very long chain compounds, one or two short transitions ($B'B''$ and BA') appear under high pressures, which, instead of leading to the solid state, give rise to a new fluid phase in which the molecules are therefore as closely packed as in the solid state. HARKINS and COPELAND[67] have given the name of *superliquid* to this phase of apparent very weak viscosity. However, as was shown by JOLY,[68] these type of fluid phases show a non-newtonian flow and their apparent viscosity depends enormously on the rate of shear.

d. Higher order transformations

The definition of higher order transformations as applied to the case of monolayers was given by DERVICHIAN and JOLY;[53] a more rigorous definition can also be found in a more recent paper by DERVICHIAN.[64] It is sufficient to say here that an ordinary transformation in which a discontinuity appears in the molecular area (e.g. the transition from V to I on Fig. 10 or from F to E on Fig. 11) is a transformation of the first order; but a discontinuity in the compressibility (i.e. a sudden change in the slope of an isotherm such as the point A in the figures) corresponds to a transformation of the second order. Similarly, a change in the slope of the curve representing the compressibility as a function of the surface pressure would be a transformation of the third order.

Isotherms of the shape represented in Fig. 14 were known for a long time. The change of slope which occurs at the point A is evidently due to a transformation of the second order which corresponds to the passage from the mesomorphous phase to the solid phase without the occurrence of any discontinuity in the molecular area. Other transformations of higher order, which were recognized and studied systematically for the first time by DERVICHIAN and JOLY,[53], [58] occur in monolayers. These transformations produce very distinct changes in the surface viscosity. Furthermore, by recording the isotherms automatically and in a continuous way, it became possible to detect safely discontinuities in the compressibility of the monolayers which corresponded to those which had been revealed by the surface viscosity measurements.

The study of transformations of different order was resumed some time later by HARKINS, YOUNG, and BOYD[60] who, besides the determination by the study of the compressibility, tried to introduce, without great success, a determination based on the variation of latent heat of transformation.

DERVICHIAN and JOLY have arrived at the important conclusion that there is an intimate connection between ordinary transformations and higher order transformations in monolayers of the fatty substances. Thus, *when an ordinary*

transformation ceases to occur, because a higher temperature range is reached (or a different pH value), it is replaced by a transformation of a higher order which occurs for the same characteristic area per chain. For example, the area of 38–39 Å², which corresponds to the liquid phase at the triple point (see Figs. 17 and 18), is found again at a point where a higher order transformation occurs during the compression of the liquid or the gaseous phase (see point *H* on Figs. 9, 10, 11, and 12). Similarly, higher order transformations appear at the points *C*, *B'*, and *B* (see Figs. 11, 12, 13, and 14) corresponding to the areas of 23·5, 22 and 20·5 Å² at which ordinary transformations occur under vapour pressure (Figs. 14 and 15) or by compression of the mesomorphous phase (Fig. 16) either in other ranges of temperature, or with longer hydrocarbon chains.

ISEMURA and HAMAGUCHI[69] have found that these characteristic points of higher order transformation appear still more neatly when working with definite mixtures of different fatty acids.

JOLY[70] has developed a complete theory of the ordinary and higher order transformations in monolayers of the fatty substances based on structural considerations. Molecules in a monolayer can only exist in a finite number of energy states corresponding to a discrete series of molecular forms and hence molecular areas, these last being precisely the areas of points of transformation.

3. *Relations between molecular constitution, molecular area, and physical state in monolayers*

It is tempting to use measurements of molecular area in monolayers as a means of determining molecular constitutions, but this should be done with great caution. From this point of view, the comparison of the molecular areas of different compounds has a meaning only if they are considered in the same physical state. For example, a too hasty comparison of the molecular areas of myristic acid and palmitic acid at 30° would lead to the evidently wrong conclusion that myristic acid has a more bulky molecule than palmitic acid. On the contrary, a careful study of triglycerides and fatty acids in different ranges of temperature, where they are in the same states, shows that the area per chain remains practically the same (in the same state) whether the molecule contains a single chain or three hydrocarbon chains. It was shown by ADAM and DYER[71] that even when four chains are present in one molecule, as in pentaerythritol tetrapalmitate, the area per chain under high surface pressure is 20 Å².

Recently, WEITZEL, FRETZDORFF, and HELLER[72a] have examined the complete series of 2-methyl, 3-methyl, 4-methyl . . . palmitic and stearic acids and have found that the molecular area under vapour pressure, as well as the form of the isotherm, varies with the position of the substituted methyl group. Actually, the layers are more or less condensed and the form of the isotherms, at a given temperature, varies from the type of Fig. 14 to that of Fig. 10. An examination of the melting points of these series of substances, which are given in another paper by WEITZEL and WOJAHN,[72b] shows that their rather irregular variation with the position of the substituted methyl group is strikingly parallel to the variation of state, at a given temperature, in the corresponding monolayers.

Obviously the position of the substituted CH_3 group has an influence on the molecular interactions, but it is not possible to say if it changes the size or at least the packing of the molecules, unless areas are compared in the same state and therefore after a study in a wide range of temperature.

The study of the series of disubstituted acetic acids $\begin{matrix} R_1 \\ R_2 \end{matrix}\!\!>\!\!CHCOOH$ by STENHAGEN[73] is more conclusive. Here in the series with a total number of 17 carbon atoms, all the monolayers are in the liquid state at room temperature and the isotherms shift gradually towards larger areas when the two substituted chains tend to the same length, one increasing from C_1 to C_7 and the other shortening from C_{14} to C_8. With the long substituted chains the minimum molecular area at the point of collapse is 55–56 Å², which is consistent with the area of 27–29 Å² per chain found at the same point with liquid monolayers of the normal acids or triglycerides. This coincidence shows that the two chains are packed parallel to each other.

In the case where one of the substituted groups has only one or two carbon atoms, it is difficult to arrive at a clear conclusion, since the apparent difference of area with the corresponding normal long chain acid is mainly due to a change of state. It is also difficult to conclude from the data given by SOBOTKA, ROSENBERG, and BIERNBAUM[74] concerning monolayers of omega branched fatty acids containing from 16 to 24 carbon atoms.

The same general remarks can be applied to the results obtained by ADAM[75] with the series of esters of palmitic acid with alkyl chains having from 1 to 16 C atoms. With cetyl palmitate and octyl palmitate, conditions are such that it can be concluded from the large value of the molecular area that both the alkyl and the acid chains are directed upwards. The fact that with the shorter alkyl groups the films are of the very expanded gaseous type is consistent with the low melting points of these esters as compared to the fatty acid, and indicates a weak cohesion between molecules.

This relation between the ordinary melting point of a substance, which is an indirect measure of the cohesion forces between its molecules, and the state of the monolayer it gives at a given temperature, appears again in the results obtained by MARSDEN and RIDEAL[76] with different *cis* and *trans* unsaturated compounds. These authors have found that *trans* compounds give more condensed layers than the *cis* compounds. It should be noted, however, that if at room temperature the isotherms of oleic, elaidic and stearic acid differ and are respectively of the type of Fig. 10, Fig. 11, and Fig. 14, there is practically no difference in the molecular areas of these three acids if they are compared in the same state.

Monolayers of lecithin and hydrolecithin were examined by ADAM and JESSOP[57] at 16° and 14·5°. It appears clearly from the isotherm that lecithin gives a film which remains liquid up to the highest pressures (Fig. 10). The area per molecule under vapour pressure is 114 Å². This corresponds to an area per chain of 57 Å² which is of the same order as the values given by fatty acids in

the same state. The area per chain found at the same point with a similar phosphatide by DESNUELLE, DERVICHIAN, and MOLINES[46] is 50 Å². These authors have recorded the isotherm up to a pressure higher than 50 dyne per cm, where the film collapses. At this point the area per chain is *ca.* 27 Å² which again is equal to the area found, at this point and in this state, with the other fatty substances. With hydrolecithin, both chains are saturated and the isotherm established by ADAM and JESSOP is clearly of the expanded mesomorphous type (Fig. 12). Under vapour pressure, the area per chain here is 26 Å² and, under 20 dyne per cm, *ca.* 22 Å². As noted by the authors, at about 28° the monolayer of hydrolecithin expands and the isotherm becomes similar to that of lecithin. Thus, despite the presence of the complex polar group of phosphatides, the molecular area is mainly dependent on the hydrocarbon chains. The chemical function simply changes the range of temperature at which a particular state exists.

Similar results have been obtained with the monoglycerides by ADAM, BERRY, and TURNER.[77] At rather low temperatures, monopalmitin and monomyristin give isotherms of the type of Fig. 11. However, with these compounds, the areas under vapour pressure (65–68 Å²) and at very high pressures (23 Å²) are significantly larger than with the other long chain compounds.

4. *Relation between layer and substrate*

The bonding between the molecules of the layer and the molecules of the underlying water is one of the first questions to consider. By an ingenious method, MERIGOUX[18] has examined the flow of a sheet of water under a monolayer of oleic acid and has demonstrated that the molecules of the layer are anchored to the molecules of water and that the observed friction corresponds only to the viscosity of the water just under the monolayer. Not less ingenious was the device used by SCHULMAN and TEORELL[78] in which, on the contrary, water is carried by an expanding layer of oleic acid. They have thus shown that the mean thickness of the carried sheet of pure water is of the order of 0·03 mm.

The interpretation of the surface viscosity data led JOLY[79] to the same conclusion: what is measured is the resulting effect of the viscosity proper to the monolayer and of the friction between the water molecules which are carried by the movement of the monolayer.

Another type of relation between monolayer and substrate is expressed by the importance of the solubility of the polar group of the fatty substance in the water. There is no doubt that the greater the affinity of the polar group for water, the higher is the surface pressure which a fluid monolayer can withstand without collapse. Thus, films of triglycerides or esters in the fluid state collapse at pressures which are below 20 dyne per cm, while fluid monolayers of the fatty acids can be compressed up to *ca.* 30 dyne per cm and still higher on an alkaline substrate (i.e. when the solubility is enhanced). Lecithin, with a particularly soluble polar group gives monolayers which can withstand surface pressures higher than 50 dyne per cm.

As would be expected with the fatty acids, ionization increases the solubility

and therefore has an action on the surface pressure for a given molecular area in the monolayer. In other words, a change of the *p*H of the underlying water can affect the ionization of the COOH groups and consequently modify the state of the monolayer and the form of its isotherm at the considered temperature. The conversion of condensed to expanded films by increase of *p*H was shown long ago by LYONS and RIDEAL[80] in the case of palmitic and pentadecylic acid. There is also an expansion of these films when the water is strongly acid. ADAM and MILLER[81] obtained similar results with a series of different fatty acids. More recently, EKWALL[82] has published a very complete study of myristic acid monolayers spread on 3 M NaCl solutions (to prevent solution) at a series of *p*H values ranging between 1 and 12. The complete isotherm has been recorded at each *p*H value, so that the displacement of all the characteristic points of transition as a function of the *p*H can be followed.

It is obvious that the last step in the interaction is the combination between the fatty acid and the dissolved cation to form a monolayer of soap. The reaction already occurs with very minute quantities of metallic ions when these are polyvalent. LANGMUIR and SCHAEFER[83] determined the relation between *p*H and the formation of barium and calcium soap when a fatty acid is spread on the surface of dilute barium and calcium salt solutions, by skimming the surface and analyzing the collected material. Reaction begins only above *p*H 5; only half of the fatty acid is combined at about *p*H 6 and complete neutralization occurs only when the *p*H value exceeds 10. In a second paper, the same authors have extended this study to other metallic ions, showing that, with aluminium, one part in 10^9 already modifies the state of a monolayer of fatty acid. This action of minute traces of metallic ions on the behaviour of monolayers was also pointed out by HARKINS and MYERS[84] and by MITCHELL, RIDEAL, and SCHULMAN.[85]

The effect of the ion concentration and especially of the *p*H of the underlying solution on the surface potential of monolayers of fatty acids is still more marked. GUYOT[86] and FRUMKIN[87] had already noted that, instead of the ordinary *decrease* of the air-water potential observed when a fatty acid monolayer is spread on distilled or acid water, an *increase* is obtained when the underlying solution is alkaline. Thus, FRUMKIN measured a maximum surface potential of − 0·085 volt with a highly compressed layer of oleic acid on distilled water and of + 0·108 volt on N/100 NaOH. A more complete study of the variation of the surface potential with the *p*H for monolayers of myristic acid was undertaken by SCHULMAN and HUGHES.[88] If the surface potential at 15° for an area of 20 Å² is considered, it appears that from a value of − 0·400 volt, which remains constant between *p*H 1 and *p*H 3, it decreases gradually from *p*H 4 on, becomes nul at *p*H 9·5 and then positive, reaching a new practically constant value of *ca.* + 0·060 volt at *p*H 10·5. In the very acid region, there is an increase of the surface potential when the *p*H decreases below 0 reaching the value of − 0·560 volt on HCl 4N. Similar results were observed with stearic acid by DERVICHIAN.[59]

The decrease of surface potential is obviously in relation with the ionization

of the COOH group of the fatty acid, and the positive value reached at *p*H 10·5 corresponds to the complete transformation into soap. Ionization can also be achieved by dilution, i.e. by expanding the monolayer so that the concentration in COOH groups is weaker in the surface phase, provided that the *p*H is not too low. In fact, with oleic acid on distilled water, a surface potential value of *ca.* + 0·060 was found by DERVICHIAN[59] in the vapour-liquid transition region. GUASTALLA[89] has also found positive values for the surface potential of myristic acid monolayers in the gaseous region.

The fate of a monolayer which is transfered from the surface of water into a solid surface is of interest. This can be investigated by using the method introduced by BLODGETT and LANGMUIR.[90] Monolayers of a fatty acid can be deposited successively on a plate of metal or glass by dipping and withdrawing vertically the plate through a monolayer spread on the surface of water. This is accomplished by maintaining the monolayer under constant pressure and determining the decrease of area which measures the quantity transferred. The structure of the multilayer thus piled up on the solid surface has been examined by different authors. There is evidently a change of state as a consequence of the change of the substrate. The multilayer crystallizes into the normal crystalline form of the substance, but a certain time lag appears with the esters during which a special "vitreous" state is retained. Details of optical, x-ray, and electron diffraction studies can be found in the papers of STENHAGEN[91] and of COUMOULOS and RIDEAL.[92] DERVICHIAN and FERREIRA-DENARD[93] have examined the transfer of fluid monolayers of triglycerides (C_6, C_8, C_{10}, C_{12}) on the surface of paraffin wax under different surface pressures from 0·1 to 8 dyne per cm. With a paraffin surface and under these relatively low pressures the film is transferred only during the dipping of the plate. The results show that the more the film is expanded the larger is the area which is removed from the surface of the water to cover a given area of the solid paraffin surface. The ratio of the two areas (area of transferred monolayer/area of the paraffined plate), which is equal to 0·92–1·0 when the molecular area is smaller than 130–140 Å^2, increases regularly with larger molecular areas reaching the value of 3 for 1000 Å^2. It seems that expanded monolayers condense by passing on the solid paraffin surface.

5. *Interactions and reactions in monolayers*

a. Interactions in mixed monolayers

It is well-known that the melting point of a mixture of fatty substances is lower than that of the pure substances. One would therefore expect that a mixed monolayer, at a given temperature, would be in a less condensed state than the monolayers of each of the pure components. Indeed it was shown by COLLET[94] in 1922 that an increase of the molecular area was produced by mixing different pairs of triglycerides. With mixtures of trimyristin and tripalmitin the increase in molecular area reaches a maximum of 14% for the proportion of 1 to 1. The maximum of increase is 35% with a mixture of 4 mol of tribenzoin for 1 mol of trimyristin. An increase of area as compared to the sum of the areas of the pure

components measured separately was also found by SCHULMAN and HUGHES[95] with mixtures of oleic acid and cetyl alcohol. Evidently, when mixed with oleic acid, which gives a liquid monolayer at any pressure, the molecules of cetyl alcohol occupy a larger area than when they are in the more condensed fluid (mesomorphous) monolayer of the pure cetyl alcohol.

However, other factors must be considered in order to understand the cases where, on the contrary, there is a decrease of the mean molecular area when two different substances are mixed. Thus, as shown by LEATHES[96] or by ADAM and JESSOP,[97] addition of cholesterol or tripalmitin to fatty acids or esters gives more condensed monolayers. MARSDEN and SCHULMAN[98] also found that an equimolecular mixture of octadecyl methyl ether and octadecyl amine gave a monolayer at *p*H 2 for which the area at a given pressure is less than what would be expected if there was simple additivity of the molecular areas. A decrease of the area of 18% was found by DERVICHIAN and PILLET[99] at *p*H 9·2 with mixed monolayers of lecithin and oleic acid. Mixtures of phosphatides with triglycerides examined by DESNUELLE, MOLINES, and DERVICHIAN[46] also show a condensation with discontinuities occurring for certain definite proportions.

MARSDEN and SCHULMAN[98], [100] have interpreted "contractions" and "expansions" in mixed films as due (in addition to Van der Waals' forces acting between the hydrocarbon portions of the molecules) to interactions (i.e. attractions and repulsions) between polar groups. Different types of interactions have thus been considered: ion-ion, dipole-ion, dipole-dipole.

b. Penetration of monolayers

If a monolayer of a substance A is maintained under constant pressure and if a very dilute solution of another substance B is injected in the underlying water, no appreciable change is generally observed. In some cases, however, when A and B are liable to interact or to "associate," a very important and definite increase of the area is obtained, similar to the change of volume which occurs when gases react under constant pressure. The increase of the area corresponds to the adsorption of the dissolved molecules of B which penetrate between the molecules of A forming a mixed monolayer. The state of the film may also change completely, turning for example from fluid to solid. The mixed monolayers thus formed, generally contain the two species of molecules associated in definite stoichiometric proportions and can support higher surface pressures than the original film. Further, the phenomenon is not due to a simple adsorption of the injected substance B, since, if alone, this substance, at the concentration used, would hardly affect the surface tension of water. If, on the contrary, the area is maintained constant, the pressure increases enormously after the substance B has been injected under the monolayer A. Finally, in other cases, the soluble substance can displace the insoluble monolayer from the surface.

These different types of interactions were first described by SCHULMAN and HUGHES[95] with pairs of substances such as triglycerides or long chain alcohols on one hand and sodium oleate and phosphatides on the other hand. The study was generalized later by SCHULMAN and RIDEAL[101] who showed that the

phenomenon was very specific. The interaction in monolayers was studied by this method for a series of substances that were known to form complexes of biological interest. The existence of stoichiometric proportions in the association was established by SCHULMAN and STENHAGEN.[102]

The formation of complexes of definite composition in monolayers has been questioned by HARKINS[103] who did not succeed in reproducing discontinuities on the isotherms obtained by compressing the penetrated films. The reasons for this disagreement were to be found only after a more precise determination of the required conditions. JOLY[104] has shown that certain definite conditions of available area and of surface pressure are necessary in order to obtain and to detect the different type of associations which do not all have the same stability. By conforming to these conditions, a great number of pairs of substances giving complex monolayers in stoichiometrical proportions were studied by DERVICHIAN and JOLY.[105] In a parallel investigation, DERVICHIAN and MAGNANT[106] found that the same pairs of substances (one soluble and one insoluble) when mixed in the proportions indicated by the monolayer technique can swell in water giving different paracrystalline or isotropic phases which remain in equilibrium with an excess of water. Similarly, it had been previously shown by COCKBAIN and SCHULMAN[107] that certain pairs of substances, which form complex monolayers, give very stable emulsions when they are mixed in the same stoichiometric proportions and utilized as emulsifiers.

c. Chemical reactions in monolayers of fatty substances

It is obvious that reagents dissolved in water may oxidize or hydrolyze fatty substances spread on the surface, as they do in the bulk; but it was important to determine whether the rates of these reactions differ when they occur on a surface, and if they vary with the state of the monolayer.

The oxidation of oleic and petroselinic acids on dilute acid permanganate was examined by HUGHES and RIDEAL[108] using the method of surface potential. Oxidation produces a very important fall of the surface potential of oleic or petroselinic acid which can be followed as a function of time, till a limiting value is reached which corresponds to the complete transformation of the layer. It was thus found that the reaction rate decreases at least tenfold when the monolayer is compressed from large areas to the smallest area. These facts were interpreted by supposing that the more the molecules are packed, the more the accessibility of the double bond to the oxidizing agent is diminished.

Similar results were obtained by HUGHES[109] in the study of the transformation of lecithin into lysolecithin under the action of different snake venoms dissolved in the water. An optimum was found at *p*H 7·3 and here also compression of the lecithin monolayer greatly decreased the rate of hydrolysis.

NASINI and MATTEI[110] have examined the action either of permanganate or of ICl on monolayers of triolein, following the reaction by the increase of area. They have also examined ozonization of the double bonds by irradiation of monolayers of triolein.[111] Recently EDA[112] has determined the rate of

oxidation in monolayers of six unsaturated acids of the oleic acid type by measuring the increase of area under constant surface pressure.

Another type of investigation concerns the saponification of long chain esters and glycerides in monolayers. With the long chain esters, ALEXANDER and SCHULMAN[113] found that the rate of hydrolysis by NaOH varies with the chain length of the alcohol radical and with the compression of the monolayer. The hydrolysis of mono-, di-, and triglycerides by sodium hydroxide has been studied by ALEXANDER and RIDEAL[114] using the surface potential method and more recently by JELLINEK and ROBERTS[115] who determined the variation of area under constant pressure. Values of the deduced velocity constants and activation energies can be found in the original papers.

V. ADSORPTION OF FATTY SUBSTANCES AT THE SURFACE OF THEIR AQUEOUS SOLUTIONS

1. *Solutions of fatty acids*

The first comprehensive study on the lowering of the surface tension of water by addition of fatty acids or alcohols is found in an article by E. DUCLAUX[116] published in 1878. From the examination of the surface tensions of solutions of the fatty acids from C_1 to C_6 by the drop volume method, this author came to the following fundamental conclusions: (*a*) The surface tension decreases with increasing concentration; (*b*) The higher is the molecular weight of the acid or alcohol, the weaker is the concentration necessary to produce the same lowering of the surface tension of water; thus less than 0·4 g of capric acid per litre of water produces the same lowering as 27% of formic acid; (*c*) The ratio of the concentrations of two fatty acids (or alcohols) which produce the same lowering remains practically invariable whatever is the lowering; e.g. the concentrations of butyric acid and acetic acid which give the same value of the surface tension are in the ratio of 1 : 10 either when a lowering to the 9/10 of the value of pure water is obtained or the 6/10.

The surface tensions of aqueous solutions of different organic substances were measured thirteen years later by TRAUBE[117] using the capillary rise method. With a given acid, the lowering of the surface tension is far from being proportional to the concentration. However, examining the quotient of the lowering by the corresponding concentration, TRAUBE found that it tends to a limiting value when the concentration decreases, i.e. the lowering of the surface tension tends to become proportional to the concentration for very small values of the lowering. He also remarked that the coefficient of this proportionality is multiplied by a factor of *ca.* 3 for every increment of one carbon atom in the series from C_1 to C_5. The name of TRAUBE has since been give to this rule. Similar measurements with the acids from C_1 to C_6 were repeated by SZYSZKOWSKI [118] who proposed an empirical logarithmic relation between concentration and surface tension lowering. The surface tensions, at two different temperatures (25° and 35°) of concentrated aqueous solutions of the same fatty acids were measured by DRUCKER.[119] Solutions of nonylic acid were also examined

by DONNAN and BARKER[120] with the aim of testing the validity of GIBBS's adsorption equation.

In his fundamental paper of 1917 on monolayers, LANGMUIR[121] introduced the idea that the excess of substance, which, according to the equation of GIBBS, is concentrated in the surface region of a solution, constitutes in fact an adsorbed monolayer. Thus the lowering of the surface tension due to this adsorption is identical to the surface pressure produced by monolayers of the insoluble substances. FRUMKIN[122] measured the surface tension lowering of solutions of the C_8, C_{10}, and C_{12} acids and compared the results to the lowering produced by the monolayers of the insoluble fatty acids. Using FRUMKIN's results and the equation of GIBBS, SCHOFIELD and RIDEAL[123] calculated the amount of adsorbed fatty acid and found that the derived $\pi - A$ (surface pressure — molecular area) isotherm for lauric acid coincided fairly well with the isotherm obtained when the same acid was spread on the surface of water and studied by the monolayer technique. The surface potentials of aqueous solutions of the lower fatty acids were also measured by FRUMKIN[124] showing that they varied in a parallel way to the lowering of the surface tension. BOUHET[15] examined the ellipticity of the light reflected from the surface of the aqueous solutions of the series of the fatty acids. This ellipticity varies continuously with the concentration reaching a limiting value when the solution is saturated. The limiting value, which is negative, increases regularly with the chain length. Examining also the surface of water covered by an insoluble monolayer of palmitic acid, BOUHET showed that the value of the ellipticity found in this case falls on the extrapolated curve plotted with the values of the soluble acids from C_3 to C_{12} as a function of the chain length.

Using a new method, DERVICHIAN[125] has measured directly the surface pressure of films of the C_8, C_9, C_{10}, and C_{11} normal fatty acids adsorbed at the surface of their aqueous solutions, as a function of the concentration. For all these acids, the surface pressure is proportional to the concentration in the solution for the very weak concentrations and as long as the surface pressure is lower than 0·5–0·6 dyne per cm. Then, the pressure rises much more rapidly with the increase of concentration. As was discussed by LANGMUIR in the above-mentioned paper, such a result is to be expected. As noted by different authors, a certain length of time is necessary for the adsorption to reach an equilibrium. This is reflected at first in a rapid and then in a progressively slower decrease of the surface tension in the course of time, from the moment of formation of the surface to the attainment of the equilibrium surface concentration. With the fatty acids, equilibrium was practically reached after nearly 1 hr.

From the results of DUCLAUX and TRAUBE and those which have since been accumulated, it can be concluded that, as a first approximation, the amount of fatty acid which is needed to produce a certain lowering of the surface tension is very roughly proportional to its solubility. In other words, the same order of magnitude for the surface concentration of adsorbed molecules is obtained with the different fatty acids when their respective concentrations in the solution are such that the thermodynamical activities are equal. However, the surface

tension is not simply related to the surface concentration, since, as was shown in the study of monolayers, for a given surface concentration, the surface pressure (i.e. the lowering of the surface tension) depends on the *state* of the film which itself is dependent on the chain length, the temperature, the *p*H, and the salt concentration. The solubility can be taken, nevertheless, as a first rough indication to predict the surface activity of the dissolved substance.

It is therefore to be expected that a change of the *p*H, which changes the solubility of a fatty acid, will strongly affect the surface tension. The formation of soap, which is much more soluble in water than the fatty acid, can be considered as the extreme case attained when the *p*H is brought above *p*H 10 or 11. Thus, at 25°, less than $7 \cdot 10^{-6}$ mol per litre of lauric acid is necessary to produce a lowering of 20 dyne per cm (equilibrium value) of the surface tension of acid water, but in order to obtain the same lowering at *p*H 11, *ca.* 10^{-3} mol of potassium laurate is needed, that is at least a hundredfold more.

2. *Solution of soaps*

The action of soaps on the surface tension of water has been known for a long time. The first systematic study of the surface tension of the aqueous solutions of the sodium salts of the series of the normal fatty acids is due to LASCARAY.[126] With the exception of Na formiate and acetate, which produce an increase, a steady decrease of the surface tension is observed when the concentration of the soap is increased. The surface activity increases with the chain length, i.e. the higher is the molecular weight of the acid, the less is the concentration of soap needed to produce a certain lowering. At room temperature, at which these determinations were done, a maximum of the surface activity was found with Na-myristate. With longer chains, such as Na-palmitate and stearate, the lowering, at equal concentration, was less than with Na-myristate, and with Na-cerotate (C_{26} acid) no lowering was practically observed.

These peculiarities are evidently the consequences of the following facts: (*a*) at room temperature, the sodium salts of the higher fatty acids are below their temperature of complete solution in water (Krafft point, see p. 211); and (*b*) the adsorbed layers with the very long chain acids are of the solid type, therefore brittle and unable to support the increase of area implied in the measurement of surface tension. In fact, a steady increase of the surface activity with the chain length, without any maximum, is observed at higher temperatures as can be seen in the work of LOTTERMOSER and TESCH[127] who examined the solutions of these different soaps at 20° and 65°. The same reasons should be given to explain the results of HIROSE and SHIMOMURA[42] who found that, at low temperature, Na-stearate solutions showed a higher surface tension than the unsaturated C_{18} fatty acid soap, while the contrary obtained at high temperature. CAVIER[128] has shown that the higher is the degree of unsaturation of the fatty acid, the lower is the surface tension of the corresponding soap solution.

Besides the length or the nature of the hydrocarbon chain of the fatty acid and the temperature, the *p*H and the salt concentration of the soap solution have a very marked influence on the surface activity. HARKINS and CLARK[129] had

found that the addition of NaOH to a solution of 0·1 M sodium nonylate raised the surface tension from 20 to 48 dyne per cm. Similar effects were observed by LOTTERMOSER and TESCH[127] by adding either NaOH or Na_2CO_3 to solutions of different soaps. POWNEY[130] drew attention on the fact that small changes of *p*H can produce very considerable changes of the surface tension of soap solutions. He also showed that the influence of CO_2 on the surface tension of these solutions can be correlated with changes of the *p*H and concluded that data which are found in the literature are of little value, unless the exact conditions of the *p*H value of soap solutions are stated. In a paper of LONG, NUTTING, and HARKINS[131] which followed, curves giving the surface tension of solutions of sodium laurate as a function of the concentration have been established for different *p*H values. It can be seen, for example, that the curve for *p*H 11·1 is above that for *p*H 8·5, indicating that the more the solution is alkaline, the more soap is needed to produce the same lowering of surface tension. Adding an alkali to a dilute solution of soap which has a low *p*H value (due to the dilution of an originally neutral concentration solution) can therefore only decrease the surface tension.

As mentioned previously, soaps undergo hydrolysis with dilution and when the *p*H is lowered. Free fatty acid is therefore present in these cases in the solution. To explain the decrease of the surface tension with decrease of the *p*H of soap solutions, ADAM[132] has therefore proposed that free fatty acid or acid soap is more strongly adsorbed than the neutral soap on account of the much greater attraction of COONa for water than COOH. Indeed, analysis of foam obtained from soap solutions by LAING[133] and more recently by RAISON[134] shows that the layer adsorbed at the air-solution interface contains a certain amount of free fatty acid.

The dependence of the surface tension lowering on the *p*H value may perhaps be easily understood by simply considering the enormous variation with the *p*H value of the solubility in water of the fatty acids. This is perhaps repeating ADAM's explanation in other terms, but it enables an understanding of the fact that, when larger and increasing quantities of alkali, neutral salts or even buffers, are added to soap solutions, the surface tension decreases slowly. If small amounts of alkali increase the solubility by increasing the *p*H, larger quantities of alkali or salts decrease the solubility, as is shown by the decrease of the critical concentration of micelle formation (see p. 211) and the final stage of the salting-out.

It was established by LOTTERMOSER and TESCH[127] that, after decreasing with increasing concentration, the surface tension of a soap solution reaches a minimum value where it remains practically constant over a certain range of concentration, and then rises to a certain extent for higher concentrations. They also found that the concentration at which the minimum value is first reached decreases with increasing chain length of the compound and that it also decreases when the solution becomes more acid or when large amounts of alkali or sodium carbonate are added. It is well known now that the minimum occurs approximately when the critical concentration of micelle formation is reached. The

value of the surface tension at the minimum also depends on the *p*H and on the quantity of electrolytes present. SHEDLOWSKY, ROSS, and JAKOB[135] have suggested that the subsequent increase of the surface tension at higher concentrations should be attributed to the presence of impurities.

If surface tension measurements are done on soap-water systems below the temperature of solubilization (Krafft point), inconsistent results are obtained which depend on the age and previous treatment. Actually, the system being in the course of precipitation is metastable. LOTTERMOSER and SCHLADITZ[136] showed that, if the determinations with the sodium soaps were made at temperatures above the melting point of the corresponding fatty acids, reproducible results were obtained. This can be explained by noting that the Krafft point of a sodium soap coincides approximately with the melting point of the corresponding acid.

If the solutions at different concentrations are prepared by simply diluting a concentrated neutral soap solution with distilled water, the *p*H value decreases with increasing dilution and variations occur in the degree of hydrolysis which are not continuous, as has been shown by different authors and in particular by EKWALL.[137] As a consequence of the intervention of this additional variable, different humps appear on the curve representing the surface tension as a function of the soap concentration. If the *p*H is maintained constant by diluting with a buffer solution of a determined concentration, these humps disappear, so that reproducible and smooth curves are obtained, as for example with potassium laurate, for all the series of hydrogen ion concentrations between *p*H 3 and *p*H 12.[138] The equilibrium value of the surface tension with soap solutions is attained only after 3 to 4 hr, particularly in the region of weak concentrations before the minimum point. This fact has been noted by different authors and had already been described by RAYLEIGH in 1890.

VI. Adsorption of Fatty Acids at the Solid-Liquid Interface

Although, for numerous substances, the general problem of adsorption on solids from solutions has received much attention, publications on the adsorption of fatty substances at the solid-liquid interface are rather scarce.

BARTELL and FU[139] examined the adsorption by silica of the organic acids from C_1 to C_4 from their solutions in carbon tetrachloride. At a given concentration, the amount adsorbed decreases when the chain length of the acid increases. The adsorption on titanic oxide of oleic acid dissolved in benzene was investigated by HARKINS and GANS.[140] Adsorption increases with the concentration, but becomes practically constant when the concentration of *ca.* 0·02 mol of oleic acid per kilogram of benzene is reached. From the examination of the data on the heat of immersion, these authors conclude that the polar groups of the oleic acid molecules are oriented towards the oxide at the solid liquid interface.

In contrast to silica, carbon exhibits a higher degree of adsorption from aqueous solutions than from solutions in organic liquids. LINNER and GORTNER[141] have examined the adsorption of the C_1 to C_6 acids from their aqueous solutions, using a decolorizing carbon "Norite." Adsorption increases regularly

with concentration and with the chain length of the fatty acid. A branched chain in the molecule has little effect on the maximum adsorption of an acid, but, if the number of carboxyl groups is increased or if a hydroxyl group is introduced, adsorption decreases greatly.

Adsorption of the shorter fatty acids from their aqueous solution on different samples of activated charcoals has been determined more recently by LEMIEUX and MORRISSON.[142] Although, for a given concentration, adsorption is more important for a longer chain, the maximum amount which can be taken up decreases markedly in the series from acetic acid to valeric acid. Thus, assuming that adsorption is in the form of a monolayer, it is found that the available area on the charcoal varies both with the used sample and with the adsorbed substance. According to these authors, the differences are due to the diameter of pores which has to be considered in view of the chain length of the acid.

The adsorption, from solutions in benzene, of lauric acid on barium sulphate and of nonylic and oleic acid on cinnabar were examined by HELD and SAMOCHWALOV[143] and by HELD and KHAINSKY.[144] From microscopic determination of the size of the particles, the total area was evaluated, and the authors concluded that a double layer of the adsorbed molecules is formed on the solid surface.

These authors have also studied the adsorption of the sodium salts of oleic and nonylic acids from their aqueous solutions on barium sulphate, cinnabar, and kaolin. A maximum of the adsorbed quantity is reached for a certain concentration in water; with our present knowledge this can be said to correspond approximately to the critical concentration for micelle formation. According to these authors, a double layer is adsorbed on the solid surface. Recently CORRIN, LIND, ROJINSKY, and HARKINS[145] have studied the adsorption on graphite of potassium myristate dissolved in water. The area of the graphite powder was determined independently by measuring the adsorption of nitrogen. This enabled a calculation of the minimum area, at 35°, per molecule of the adsorbed myristate, which was 36 Å^2. Maximum adsorption occurred for a concentration in the soap solution of 0·01 mol. A decrease of the adsorption was found with higher concentrations.

In connection with the influence of *p*H on the adsorption, PHELPS and PETERS[146] have measured the quantity of substance adsorbed on charcoal from aqueous solutions of propionic and caproic acid at different *p*H values. They have found that adsorption decreased when the *p*H increased above the value of 5 and was practically nil at about *p*H 7. The conclusion was drawn from these experiments that only the undissociated form of the fatty acids is adsorbed. It should be noted, however, that the authors have examined in all cases the same weak concentration of fatty acid (2 g per litre) and that, with the sudden increase of solubility which occurs above *p*H 5, this concentration is too small to show any appreciable adsorption. It would be desirable to make a systematic study with varying concentrations for each *p*H value in order to settle this question.

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5

UREA INCLUSION COMPOUNDS OF FATTY ACIDS

Hermann Schlenk

Introduction

In discussing the history of the urea inclusion compounds, Bengen relates that the accidental observation that fat separates out from milk upon addition of urea suggested a possible method for the quantitative determination of milk fat. In a series of experiments to this end, a few drops of n-octyl alcohol had been added to one of the samples to achieve better separation. After the solution had stood for a while, crystals formed in the interphase. Analysis showed that urea and some organic compound were the only constituents of these crystals. Tests using solutions of urea and n-octyl alcohol without milk yielded the same compound. By varying the organic reactant, similar crystals were obtained, always composed of urea and an aliphatic component. Although positive with a great variety of aliphatic compounds, among them n-octane, the reaction failed with iso-octane. Bengen found that the formation of urea compounds with straight chain but not with branched chain molecules is a general principle and can be used for their separation.[45] He prepared the crystalline urea adducts of hydrocarbons, alcohols, aldehydes, ketones, acids, and esters and concluded that the prerequisites of the otherwise unlimited variations of the reaction are: (1) the molecules must have more than 4 to 6 carbon atoms, and (2) the carbon atoms must be linked to form a straight chain. These observations were summarized in a patent application entitled "Method for Separation of Aliphatic Oxygen-containing Compounds and of Straight Chain Hydrocarbons of at least 6 Carbon Atoms from Mixtures Containing Them."[1a] Soon afterwards, in 1940, Bengen transferred his rights to the former *I.G. Farben-Industrie A.G.*

This discovery was further developed by the Oppau Laboratories of the *Badische Anilin & Sodafabrik* which was then a part of the I.G. Farben organization. In their laboratories, W. Schlenk Jr. investigated intensively the reaction of urea with aliphatic compounds to determine the chemical nature of the adducts and possible practical applications. The structure of the crystals was elucidated in co-operation with C. Hermann of the Crystallographic Institute of the University of Marburg. The findings were restricted to this group until the German patent application and subsequent findings became available and were transferred to the Allies.[1b] The research departments of the *Standard Oil Company* (Zimmerschied, Dinerstein, Weitkamp, and Marschner) and of the *Shell Development Company* (Fetterly, Redlich, Gable, Dunlop, Millar, and Smith) were the first laboratories to follow the

study of this surprising phenomenon. The fundamental publications, appearing in 1949–1950, drew general attention to the new field.[30], [11], [59] Since then the number of reports on urea inclusion compounds has rapidly increased, a considerable part of them being devoted to fatty acid compounds.

Virtually all naturally occurring fatty acids form adducts with urea. Up to the present time, of the various publications on urea compounds, those on fatty acids attract the most interest. In research on fatty acids the distinction between linear and branched carbon chain molecules, important in hydrocarbon studies, becomes less significant. Fatty acids also played a minor role in developing the theory of urea adducts, but the great variety of known structures brought to light a number of interesting new facts. A mere tabulation of applications to fatty acids or their derivatives, without giving a theoretical outline, would neglect the possibility of further development and would disregard many yet unfilled gaps in present knowledge.

The principles of inclusion compound formation are demonstrated more clearly with urea than with thiourea, desoxycholic acid or other substances forming similar compounds.[43] Knowledge of these principles stimulated the search for new inclusion compound formers and the reinterpretation of older findings to accord with the new concepts.

Theory of the Urea Inclusion Compounds

Since the adducts formed by urea with linear organic compounds contain the two partners in definite proportions, they are true compounds and not solid solutions or mixed crystals. This fact has also been confirmed by tension analysis.[41] The ratios found for a series of saturated fatty acids are given in Table 1.

Table 1. The composition of urea inclusion compounds of saturated fatty acids[41]

Acid	*Moles urea per mole acid*	*Acid*	*Moles urea per mole acid*
Butyric	4·0	Capric	8·2
Valeric	4·6	Undecylic	8·9
Caproic	5·5	Lauric	10·0
Enanthic	6·0	Myristic	11·6
Caprylic	6·7	Palmitic	12·8
Pelargonic	7·6	Stearic	14·2

The molar ratios increase proportionally with the number of carbon atoms in the aliphatic component. The carboxyl group or any other characteristic groups in a molecule have little influence on these values. Although the compounds have constant proportions in their composition, the fractional values are *not* in agreement with the law of multiple proportions. This law requires stoichiometric factors of small whole numbers which are governed by the valences and covalences of the components forming a complex. Here the factors apparently indicate the number of urea molecules which are bound by the partner according

to its chain length. For lauric acid or some others this length only incidentally happens to be a whole number multiple of the portion corresponding to the length covered by one urea molecule. An axis takes the place of the distinct centres of coordination which are the fundamental concept in the theory of complexes. It seems desirable to avoid the term "complex" for such compounds. Their stoichiometry is mainly determined by space requirements and their formation can not be attributed to the same forces which build complexes around discrete centres of coordination in the sense of WERNER's theory and its extension to purely organic complexes.*

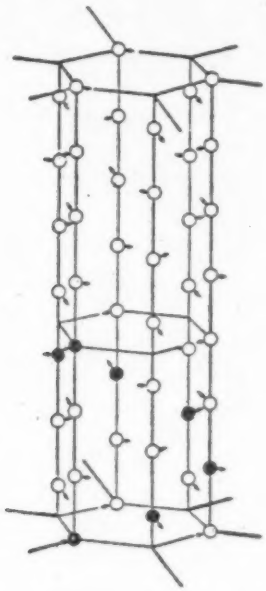


Fig. 5. The structure of hexagonal urea.
(from W. SCHLENK, Jr.[41])

All urea inclusion compounds crystallize in hexagonal prisms and generally appear in the form of thin or coarse needles (Fig. 1). Unsaturated acids and their esters tend to form short and thick crystals, even flat hexagonal leaflets being obtained from highly unsaturated ones (Fig. 2). In any case the adducts are easily distinguished from urea which crystallizes in tetragonal prisms (Fig. 3). Some urea compounds were found which do not belong to the hexagonal type. They will not be discussed here since they are mainly composed of α, ω-bifunctional aliphatics.[42]

Analysis of the crystal structure revealed that the regular hexagonal prisms are built up exclusively of urea; the six molecules forming the unit cell (Fig. 5) occupy the edges of the prism spiraling over a length of 11·1 Å. More specifically,

* In the literature on the urea reaction the following terms are found: complexes, adducts, molecular compounds, clathrate compounds, inclusion compounds and similars. The term "inclusion compounds" seems to be more correctly descriptive for this type of compounds.

the oxygen atoms are located in the edges, and the $C\begin{smallmatrix}/N\\\backslash N\end{smallmatrix}$ groups lie nearly flat in the plane between two adjacent prisms. In Fig. 5 these directions are indicated by arrows.[41], [46], [47]

Since pure urea is known to crystallize only in tetragonal form, it seems to be conditional for the stability of the hexagonal structure that a suitable partner be present. Removal of the partner by extraction or volatilization causes instantaneous breakdown as indicated by the chalky spots in Fig. 4. The x-ray pattern of the residual powder is that of tetragonal urea. The hexagonal urea pattern, however, is identical regardless of whether the adduct contains a fatty acid, an ester, a hydrocarbon, or others.

The honeycomb-like structure suggests the assumption that the aliphatic molecules occupy the free space inside the hexagonal channels. The cross-sectional diameters of molecules bound by urea fit well into the lumen of the prism. Basing such calculations upon the van der Waals packing radii, the maximum diameter of an extended linear hydrocarbon is close to 4·5 Å, whereas the inside diameter of the channel is approximately 5·5 Å. The experimental fact that molecules undoubtedly thicker than this available diameter do not form urea inclusion compounds accords with this assumption.

Two types of evidence have been found to support the concept of host molecules, the urea, building up a crystal structure which provides space for a guest molecule, the straight chain compound. The compound included must add to the weight of the housing structure without changing its volume. Actually, the specific gravity calculated from x-ray data for the hexagonal urea plus the number of guest molecules per cubic centimetre are in good agreement with the values obtained directly.[41] Furthermore, discrete spots or diffuse bands are found in the x-ray pictures of some adducts and it has been possible to trace those back to the aliphatic components. These diffractions are superimposed on the unchanged pattern of hexagonal urea. The identity periods corresponding to the spots or bands are in excellent agreement with the length of the included molecule when an extended zigzag constellation is assumed.[47] These measurements have been carried out on adducts of hydrocarbons and of 1,10-dibromodecane only. For fatty acids the method may conclusively demonstrate whether the molecules are oriented carboxyl to carboxyl in the channel, as in crystals of the pure acids, or are oriented in some other way.

Between the two groups of molecules which are either reactive or inert to urea are compounds ambiguous in their behaviour. These molecules have diameters fitting tightly into the urea lattice and react only when a considerable amount of another readily reactive adduct former is present. This requirement may be fulfilled *inter*-molecularly, as has been demonstrated with a mixture of 3-methylheptane and n-decane: pure 3-methylheptane is not able to react with urea but is included together with n-decane. The condition may also be met *intra*-molecularly as in the case of octadecylbenzene. Although benzene itself does not react with urea, the long aliphatic chain in octadecylbenzene provides enough

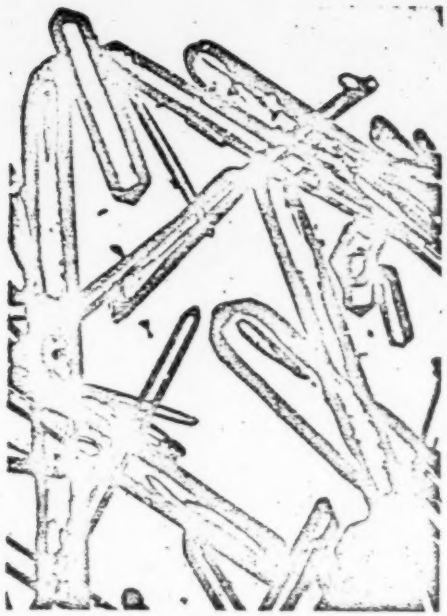


Fig. 1. Urea compound of eicosanoic acid.

Fig. 2. Urea compound of shark liver oil acids.
(from ABU-NASR[22a])

Fig. 3. Urea
(from W. SCHLENK Jr. [41])

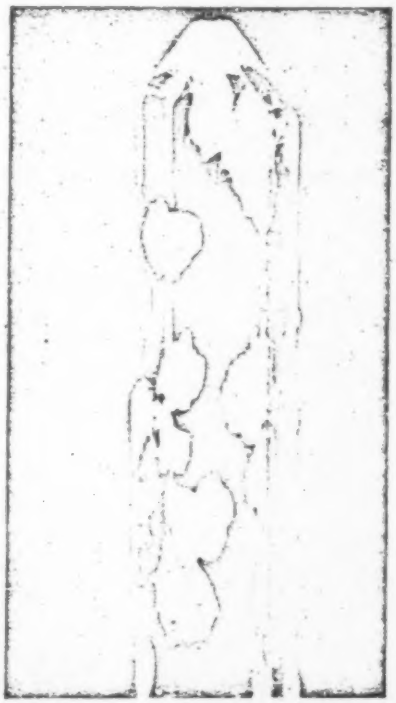


Fig. 4. Urea compound of di-n-butylamine, partially decomposed by volatilization of the amine.
(from SCHLENK Jr. [41])

(To face page 246)

energy for the urea structure formation to cover the benzene ring without the latter contributing to the stability.[41] Concluding from a considerable number of similar examples, "branched chain" and "straight chain" do not exhaust the classification of the urea reaction. Even straight chain compounds may offer difficulties in the adduct formation, as might be expected for iodo-compounds. This effect has been shown by MEADE for some brominated fatty acids. SWERN and co-workers centred a study of the isomeric 9,10-dihydroxystearic acids on their diameter. Steric considerations will also play a role in research on branched fatty acids, e.g. the methyl substituted acids and the acids of Chaulmoogra oil. A more detailed discussion of this intermediate group will be given at the end of this theoretical outline.

The formation of urea inclusion compounds is an exothermic process. Caloric data on the reaction of fatty acids and esters with urea are compiled in Table 2.

Table 2. Heat of urea adduct formation
solid urea + dissolved acid or ester → solid adduct

	Calorimetric[49]	*Calculated from equilibria*[50]
Acids		
Caprylic		10·0
Undecylenic		19·7
Myristic		22·5
Oleic		27·4
Methyl esters		
Undecylenate		17·0
Myristate	16·1	
Palmitate	19·4	
Stearate	22·2	19·0
Oleate	20·6	25·0
Linoleate		24·6
Linolenate		22·5
11-Eicosenoate	21·2	
13-Docosenoate	23·7	
12-Methylmyristate	7·8	
Isopropyl esters		
Oleate	7·0	
11-Eicosenoate	15·5	
13-Docosenoate	19·5	

Since only part of these measurements have been made specifically in the interest of fatty acid chemistry, they need supplementation and correction. The following conclusions, however, can be drawn: the molar heat of formation increases with chain length, and diminishes upon introducing unsaturation in a given chain; the obstruction a methyl branch offers to adduct formation causes an abrupt decrease of the heat of reaction.

The results obtained calorimetrically and those obtained by calculation from equilibria agree insufficiently for comparison. In particular, the low value

found for methyl stearate by the latter method is in contrast to above generalizations and to other experimental findings indicative for the high stability of saturated compound adducts.

Caloric measurements carried out by W. SCHLENK Jr. served in computing the heat increments of the urea addition to different groups in the molecule.[41] In Table 3 the change of energy involved by this one step is listed, the energy

Table 3. Heat increments of the urea addition to various groups

Group	*g Cal/1 g equiv.*	*g Cal/1 Å length*
$>CH_2$ . . .	2700	2150
>C=O . . .	7900	6300
—OH (prim.) . .	4300	3000
—COOH . . .	13600	5500
—COO— . . .	8200	3400

required for converting tetragonal into hexagonal urea and the heat of evaporation being subtracted from the experimental overall values. These energies are the binding forces between urea and the molecules included. They qualitatively reflect the tendency for hydrogen bond formation of the oxygen containing groups. For the methylene group the value must represent van der Waals forces. SMITH considers 2700 cal per g equiv. CH_2 as too high and explains the stability of the adducts as due largely to hydrogen bonds between the urea molecules in the lattice.[47] Since pure urea is known only in tetragonal form, the additional forces acting between urea and the guest molecule must have the deciding share in the stability. In long chain compounds the van der Waals forces between methylene groups and urea outweigh the additional binding energy of the functional groups.

The lowered stability of unsaturated compounds, significant in work with fatty acids, remains unexplained. The irregularity in the unsaturated molecule may prevent establishing balanced optimum distances for the potential between urea and the area of the double bond. Therefore the associating forces are not as strong as for saturated chains in urea. Experiments on triple bond compounds which might check any speculation explaining the behaviour of the double bond have not been reported. A similar phenomenon, however, may be seen in the instability of straight chain thiourea inclusion compounds. In the adducts formed by thiourea the lattice has a wider lumen than in those formed by urea[16], [46] and branched and cyclic compounds are therefore readily included. On the other hand, straight chains do not pack tightly enough the available space in thiourea so that the structure is not evenly "supported" but is likely to collapse.[24], [37], [42]

The interaction between urea and specific groups of the guest molecule is also demonstrated by the separation of optical isomers from racemic mixtures by means of the urea inclusion reaction. In principle the procedure applies to all

urea adducts although being reported so far for only 2-chlorooctane.[44] In Fig. 5 the urea molecules form a right-hand threading, which cannot be brought in congruency with its mirror image. The two lattices are enantiomorphic, each form accommodating preferentially one component of the racemic mixture as a result of the localized forces between asymmetric lattice and asymmetric guest.

The dimensions of the urea unit cell are known very precisely. HERMANN found the period of identity for the urea to be 11·1 Å and the diameter of the unit cell to be 8·20 Å (cf. ref. [41]), and the values determined by SMITH, 11·0 Å and 8·23 Å respectively,[46, 47] are in excellent agreement with this. The available lumen is much less clearly defined. Although exact values are known for the distance of the centres of atoms chemically bound, they cannot be given so precisely for the van der Waals radii or the mutual penetration of atoms or atom groups. W. SCHLENK Jr. and HERMANN assume a maximum value of approximately 5·5 Å for the available diameter,[41] SWERN and co-workers assume 5·5–6·0 Å,[54] whereas SCHIESSLER and FLITTER derive 4·1 × 4·8 Å for the lumen.[37] When properly interpreted, this disagreement is not serious because molecules of about 4·5 Å diameter permit an optimum for van der Waals attraction, whereas for 5·5 Å repulsion is prevalent. This statement considers only hydrocarbons or the hydrocarbon moiety of a molecule. The experimental material does not suffice to show to what extent hydrogen bonding might compensate for thickness as there are no caloric data reported for compounds having sec. hydroxyl, methoxyl or hydroxymethyl groups or for similar series which are comparable with their straight chain isomers.

x-ray diffraction showed maximum extension for hydrocarbons and α,ω-dibromohydrocarbons included in urea and consequently regular zigzag constellation of the molecule is approached at least by them very closely. It is uncertain, though, how far the constellation preferable for substituents in an isolated molecule might be distorted to make it fit to the high relief of potential energies inside the urea structure. Taking dichloroethylene as an example of a critical fragment, such rotation changes the diameter of the molecule as is easily seen with atom sphere models. Keeping this in mind, a comparison of alternative structures for a compound will be satisfactory for a qualitative prediction of adduct formation. The Fisher-Hirschfelder-Taylor[10] or the Stuart models[21] offer the simplest approach. They demonstrate space filling and stearic hindrance according to the atom radii as they were known in 1936 and 1934. These values, besides no longer being up-to-date in many respects, were also modified in constructing the models and their use permits only a rough approximation. A comprehensive discussion of atom models in keeping with new findings has been given by BRIEGLEB in 1950.[3]

The empirical approach to find principles predicting whether a molecule will form an inclusion compound with urea has been most accentuated for variations in the skeleton of acids and alcohols. It is not to be expected that an "all or none" principle applies and there are some discrepancies in the results due to differing techniques. TRUTER,[36] whose work is of major interest, dissolved the substance to be tested in 100 parts of benzene and used for the reaction solid

urea moistened with methanol. In Table 4, the compounds are grouped according to their structure, the partitions within the groups being drawn between negative and positive response to urea. The position of the branch relative to the carbonyl of the ester group follows the numbering system

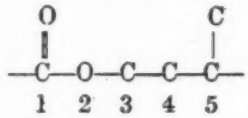


Table 4. Effect of chain length versus branching on reaction with urea

	No reaction	*Reaction with urea*
I. Methyl in position 3	1-methylheptyl acetate 1-methylheptyl propionate	1-methylheptyl butyrate
II. Methyl in position 4	2-methylbutyl propionate 2-methylbutyl octanoate	2-methybutyl decanoate 2-methylbutyl dodecanoate
III. Ethyl in position 4	2-ethylbutanol 2-ethylbutyl octanoate 2-ethylhexyl hexanoate	2-ethylbutyl decanoate 2-ethylbutyl dodecanoate 2-ethylhexyl octanoate 2-ethylhexyl dodecanoate
IV. Methyl in position 5	3-methylbutanol 3-methylbutyl propionate	3-methylbutyl hexanoate 3-methylbutyl octanoate
V. Two methyl groups	2,6-dimethyloctene-1-ol-8 (citronellol) citronellyl butyrate 2,6-dimethyloctadiene-2,6-ol-8 (geraniol) geraniol octanoate	citronellyl octanoate geranyl dodecanoate

Apparently the chain length necessary to support inclusion compound formation depends upon the position of the substituent. Taking the ester oxygen as a link equivalent to one carbon atom and cutting out the two atoms neighbouring the tertiary carbon atom, the minimum requirement is a straight chain of 8 atoms (I, IV) or 11 atoms (II) whereby two small chains are as effective as one long chain. In explaining the different requirements of 8 and 11 atoms, TRUTER points out that the side chain is closer to the side of the carbonyl for odd number substitutions than for the even numbered ones. In the latter position the substituent and the carbonyl are on the opposite side of the stretched axis of the molecule and so the diameter is increased. This is illustrated in Fig. 6, where the methyl group of 2-methylbutyl decanoate and the carbonyl group point in nearly opposite directions. Fig. 7 shows n-amyl acetate, a typical adduct forming chain, whereas Fig. 8 shows 3,9-diethyltridecanol-6 as an example for a substance which does not add urea.

The results of other authors conform with TRUTER'S findings, except for

Fig. 6. 2-Methylbutyl decanoate. The axis of the molecule is turned so that the maximum diameter is approximately in the plane of the projection.

(To face page 250)

Fig. 7. n-Amyl acetate, a molecule which readily forms a urea inclusion compound.

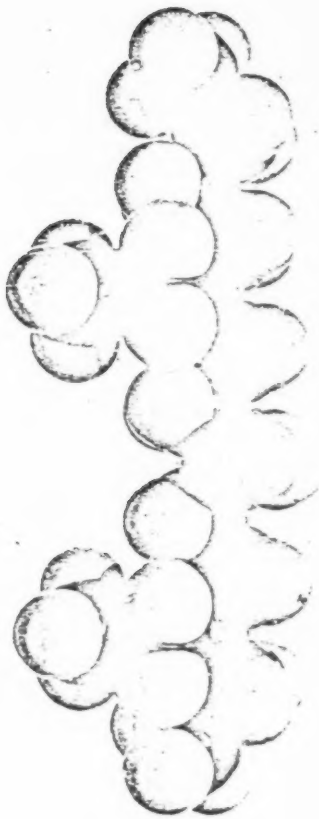


Fig. 8. 3,6-Diethyltridecanol, a molecule which does not form a urea inclusion compound.

(To face page 251)

2-ethylhexyl myristate (III) which has been reported not to form an addition compound. In listing the compounds tested, the Roman numerals indicate to which of the above groups a structure belongs. The following compounds give no reaction with urea: isobutyric acid,[41] 2-methylbutyric acid[41] (II), 2-ethylhexyl myristate[59] (III), isopropyl 12-methylmyristate,[59] the formates, acetates and toluene sulphonates of 12-hydroxystearic acid and methyl ricinoleate,[25] the oximes of ketostearic acids,[25] cholesteryl esters,[56] 3,9-diethyltridecanol,[56] triglycerides. However, the isopropyl esters of oleic, 11-eicosenoic and 13-docosenoic acid[59] (I), ethyl 3-methylundecanoate[22] (I), the methyl esters of 12-methylmyristic,[59] and 13-methylpentadecanoic acid[25] and 12-methyltetradecyl acetate[59] react with urea. Whereas 2,2-difluorooctane forms an adduct without difficulty,[59] the large bromine atom has marked influence upon the reaction. For example, 2-bromooctane,[59] 9,10-dibromostearic acid,[25] linoleic acid dihydrobromide,[25] tetrabromostearic acid,[25] dibromopalmitic acid[25] do not react with urea. However, urea adducts form with 1-bromohexane,[59] dibromooleyl alcohol[56] and 9- and 10-bromostearic acid.[25]

Rules similar to those discussed above for branched esters are found in TRUTER'S publication for molecules having benzene and cyclohexane as the critical area. Benzoic acid esters apparently hold a special position in that they could not be brought to reaction at all.

PREPARATION AND ANALYSIS OF PURE UREA COMPOUNDS

Urea binds fatty acids and their derivatives in a ratio of approximately 3 : 1 by weight. In preparing the adducts it is often necessary to apply an excess of urea in order to obtain a satisfactory yield from the equilibrium,

$$x\text{Urea} + y\text{Acid} \rightleftharpoons \text{Urea}_x \,.\, \text{Acid}_y.$$

Equilibrium constants for several fatty acids and esters at different temperatures have been measured by REDLICH and co-workers.[30] The conclusions in discussing the heat values of the reaction were partly based on them. H. SCHLENK and HOLMAN arrived at a similar picture of relative stabilities by comparing the yields obtained from different fatty acids when the reactions were carried out under standardized conditions. Long-chain fatty acids give high yields and unsaturation opposes this. Moreover, it was found that for unsaturated fatty acids conjugation facilitates the addition compared with the nonconjugated isomers, and that *trans* configuration is favoured above *cis* configuration.[40]

To obtain a pure adduct it is advisable to bring both fatty acid and urea in solution as described in the following: 1 g of fatty acid or of a derivative is dissolved in 30 ml of hot methanol containing approximately 5 g of urea which corresponds to a solution saturated at room temperature. If necessary, chloroform is added dropwise to achieve complete dissolution of the lipid. Upon cooling to room temperature or below, a precipitate forms. It is advisable that the precipitate be checked under the microscope for unreacted fatty acid or urea. Most compounds are stable enough to be washed on the Buchner funnel with cold benzene, ether, 2,4,4-trimethyl-pentane or low boiling petroleum ether.

These solvents are inert to urea and when used cautiously they do not markedly extract the fatty acid or other lipid component. Excess urea can be removed only by recrystallization which means a complete dissociation and new formation of the adduct from the approximate stoichiometric amounts gained in the first crystallization. Isopropyl alcohol is usually a satisfactory solvent. SWERN recommends methanol or isopropanol, one-half up to two-thirds saturated with urea for the recrystallization.[20]

Variations of temperature and concentration are necessary when the tendency to form the adduct is very low. Linoleic acid, for example, does not respond at room temperature to these conditions. It can be converted in 90% yield into the urea compound by using less solvent. To the hot mixture of 7·5 g linoleic acid and 30 ml methanol, 22·5 g finely powdered urea, about the theoretical amount, is added under stirring and further heating. The solution does not become homogeneous but the adduct crystallizes uniformly by cooling slowly to − 1°.[40]

Methanol is generally accepted as the best solvent for small scale work. For large preparations it is important that the reaction takes place without any solvent, although small amounts of a urea solvent are desirable to catalyze the reaction. Like any urea solvent, a lipid solvent changes the equilibrium towards dissociation. W. SCHLENK Jr. thus found complete dissociation at room temperature for 4·65 g n-heptane inclusion compound (equal to 1 g heptane, 0·01 mol) in 89 ml benzene (1 mol), whereas dissociation is only 3·3% for 9·65 g hexadecane adduct (2·26 g hexadecane, 0·01 mol) in the same amount of benzene.[41] When the fat solvent itself is an adduct former, its equilibrium with urea is in competition with the desired reaction. Therefore, higher hydrocarbons or acetone are mostly avoided in forming the product.

The presence of urea does not interfere with the usual titration of fatty acids in methanol or ethanol using phenolphthalein. Saponification and iodine numbers have also been determined directly.[20] In saponifications urea shows a small blank value which must be subtracted. If no characteristic group is accessible to direct determination, resort must be made to quantitative recovery of one of the components or to elemental analysis. Although the molar or weight ratios are of little significance for the organic component (Table 5), they ensure the purity of the adduct isolated.

Urea inclusion compounds of the following acids and esters have been described:

*Saturated fatty acids without substituent**: Butyric$^{41}_{41}$, valeric^{41}, caproic^{1,20,41} enanthic$_1$, caprylic$^{1,20,30,40,41}_{20,22,41}$, pelargonic$^{20}_{1}$, capric$^{20,40,41}_{20,22}$, undecylic^{41}, lauric$^{20,40,41}_{20,41}$ tridecylic^{20}, myristic$^{20,30,40,41}_{20,22,41,59}$, palmitic$^{1,20,40,41}_{20,41,59}$, stearic$^{1,20,31,40,41}_{20,30,41,59}$, arachidic^{40} behenic^{40}.

Saturated fatty acids with substituent: 12-hydroxystearic^{20,31}, 12-ketostearic^{20} 9,10-dihydroxystearic (m.p. 95°)$^{20,54}_{20,54}$, 9,10-dihydroxystearic (m.p. 131°)$_{20,54}$ 9,10-epoxystearic (*cis*, m.p. 59°)^{20}, 9,10-epoxystearic (*trans*, m.p. 55°)^{20}.

Unsaturated fatty acids: 10-undecenoic$^{1,20,30}_{30}$, oleic$^{1,20,30,31,40}_{20,30,59}$, elaidic$^{20,40}_{20}$

* The superscripts refer to acids, the subscripts refer to methyl esters, sometimes ethyl esters.

petroselinic[40], petroselaidic[40], 11-eicosenoic$_{59}$, erucic$^{40}_{59}$, brassidic[40], linoleic$^{40}_{30}$, 10,12-linoleic[40], linolenic$^{40}_{30}$, α-eleostearic[40], β-eleostearic[40], pseudo-eleostearic[40], ricinoleic[31].

Brominated and branched fatty acids are listed on page 251.

Table 5. Composition of various urea compounds

	C_{18}—*Acids**			C_{18}—*Methyl esters*†	
	Adduct A.N.	*Urea : acid mole ratio*	*Urea weight %*	*Urea : ester mole ratio*	*Urea weight %*
Stearic	49·1	14·3	75·1	14·8	75
Oleic	50·9	13·7	74·3	14·5	74
Linoleic	51·4	13·1	73·7	14·2	74
Linolenic	51·3	13·6	74·6	13·7	74
Other esters‡					
Methyl palmitate		13·5	75		
Vinyl palmitate		15·1	76		
1-Monopalmitin		17·1	76		

* Calculated from A.N. of adducts.[40]
† Calculated from change of concentration of reactants.[59]
‡ Calculated from saponification or iodine number of adducts.[59]

Preparation of Urea Compounds from Mixtures and Recovery of the Fatty Constituents

The adduct formed from a mixture of potential reactants consists of a single solid phase and the "mixed compounds" have the uniform structure of the hexagonal urea without distinction of the various molecules included. To accomplish complete precipitation the amount of solvent is kept small and in general the procedure is adjusted to the character of the mixture without difficulty. The following describes a nearly 100% conversion of a fatty acid mixture into the adduct. Six hundred millilitres of methanol, previously saturated with urea at room temperature, were heated with 750 g of urea on a steam bath. Soybean fatty acids, a total of 253 g, were added in portions under stirring and continuous heating. The reactants did not homogenize, but formed three phases before final crystallization. Eventually a rather solid mass of crystals formed which was freed from the mother liquor by pressing in a Carver laboratory press. The solids, after powdering and washing with cold benzene, accounted in their analysis for 97% of the acids, while nearly 3% of them was found in the combined liquids.[40]

Coconut fatty acids react very readily without solvent due to their content of lower fatty acids. Inhibitors, reported for certain petroleum fractions[59] are not encountered in natural fatty acid mixtures. Nevertheless it takes sometimes a long period before the reaction takes place. Reaction can be hastened by adding a little methanol, but still more methanol is desirable to facilitate handling of the product.

Precipitation of natural mixtures is carried out chiefly for the purpose of some type of fractionation. When the objective is the separation of straight chain compounds from heavily branched ones, an excess of urea hardly influences the results except by mechanically retaining some of the branched components. MEADE reacted the wax esters of sperm oil by grinding 100 ml of oil with 300 g of urea wetted with 10 ml methanol. The dry powder which had formed was treated with cold petroleum ether which extracted 23 g glycerides, leaving behind the mixture of adduct and excess urea.[25]

When mixtures containing straight chain molecules exclusively are to be fractionally precipitated, the amount of urea is usually taken as the variable regulating the fraction to be bound. In several experiments soybean fatty acids were reacted with insufficient amounts of urea and the solvent then was evaporated. Under such conditions all urea had been utilized for binding fatty acids.[39] For a variety of oils, ranging in iodine number between 19 and 169 urea utilization was decreased at room temperature to about 80% when two or three volumes of methanol per weight of oil were present. The influence of the temperature on the amount precipitated was shown when 25 g of soybean fatty acids were reacted in 750 ml of methanol containing approximately 120 g of urea. In successive filtrations at 27°, 4°, and — 15°, the acids bound amounted to 5·9, 16·4, and 2·2 g. Adducts can be also obtained from concentrated aqueous solutions of urea, provided the material contains saturated or monounsaturated acids. Although the equilibria are most unfavourable in water the high concentration of urea prevents complete decomposition of the more stable adducts. The possibility of using aqueous urea solutions is mentioned several times in the literature, but only SAKURAI reported such procedure in studying the segregation of fatty acids: the acids as such and in organic solvent were reacted with aqueous urea. The latter seems to be less desirable.[36] In other experiments, an attempt was made to re-use the aqueous urea solution in a cold–hot cycle. Adduct was formed at room temperature from coconut fatty acids and aqueous urea; the oily phase of unreacted acids was then removed from the mixture. The crystals suspended in the aqueous phase were decomposed by heat and the newly formed oil was again separated. The urea solution formed new adduct when recombined with the unreacted acids.[38] FETTERLY announced recently a study of the two-phase reaction in regard to the fractionation of saturated and unsaturated acids.[9]

The fatty acids can be recovered easily from the adducts by splitting the compound with water or dilute acid and by subsequent separation. Unsaturated acids tend to give emulsions. An alternative for the recovery is the extraction of the organic component, i.e. using a lipid solvent instead of a urea solvent. MEADE extracted wax esters bound in urea by boiling twice with benzene,[25] but the author found that the extraction of palmitic acid was not complete after several hours treatment with ether in a Soxhlet apparatus. Evaporation, the most direct method of recovery for both components, is not applicable to the higher fatty acids. A cyclic process has been developed for urea fractionation of lower hydrocarbons.[8]

Properties of the Inclusion Compounds

Temperature of dissociation

The adducts, even those obtained only under special precautions, are perfectly stable as long as the compound included is not volatile and contact with any solvent for either component is avoided. These adducts are as colourless as the parent substances, but pentadecyl-diazomethylketone and 1-palmitoxy-3-diazoacetone, being yellow themselves, maintain this colour with urea.[38] When observed in the usual manner at the breakdown of the crystalline structure, all melting points of individual compounds are between 132° and 134°, which is practically the same as that of pure urea. A method of characterizing the compounds and thereby the fat components has been developed by Swern and co-workers.[20] They find the "temperature of dissociation" to be characteristic for individual fatty acid or ester adducts. This point is specified as the temperature at which the clear transparent crystals become opaque and milky. It is markedly lower than 132° for many fatty acids and it is well defined over a range of 1°. For the determination a Kofler micro hot stage is attached to a microscope (magnification 24 to 40×) and the crystals are placed on a polished copper strip $\frac{1}{32}$ in. thick. The temperature is raised not more than 1° in 5 min within 5° of the point of dissociation. Using this technique the point is recorded where definite milkiness of the formerly transparent crystals is observed. The values are reproducible within ± 1·5° (Table 6).

Table 6. Dissociation temperatures of adducts (°C)

	Acid	*Methyl ester*		*Other esters*
Caproic	64		Vinyl pelargonate	52
Caprylic	73	55	Vinyl laurate	79
Pelargonic	80·5		Vinyl palmitate	113
Capric	85	67	Vinyl stearate*	
Lauric	92·5	77·5	1-Monopalmitin*	
Tridecanoic	96		1,3-Dipalmitin*	
Myristic	103	96		
Palmitic	114	118		
Stearic	126	(132)		
Oleic	110	110		
Elaidic	116	125		
10-Hendecenoic	90			
9,10-Epocystearic, *cis*, m.p. 59°	118			
9,10-Epoxystearic, *trans*, m.p. 55°	125			
9,10-Dihydroxystearic, m.p. 95°	107	120		
12-Hydroxystearic, m.p. 81°	125	114		
12-Ketostearic, m.p. 81·5°	115			

* Crystals of adduct were opaque, and therefore technique could not be employed.

Since identification and recovery of the original material is often important, the simple preparation of the urea compounds offers great advantage over the use of derivatives. Dissociation temperatures of low melting compounds, at least in the saturated series, are in a convenient range and they are spread wider

apart than the melting points of their common derivatives (Fig. 9). The use of this method to characterize unsaturated acids besides oleic and elaidic acid has not been reported yet. For highly stable adducts, the opacity coincides with the melting of urea. This point, however, is approached in a nearly straight line for a homologus series. Since the forces building the crystalline structure increase by a constant value for each additional methylene group, the energy required to break the association also increases steadily. The features of polymorphism and alternating melting points, exhibited by even- and odd-numbered fatty acids are eliminated. Such group qualities are lost when the molecules

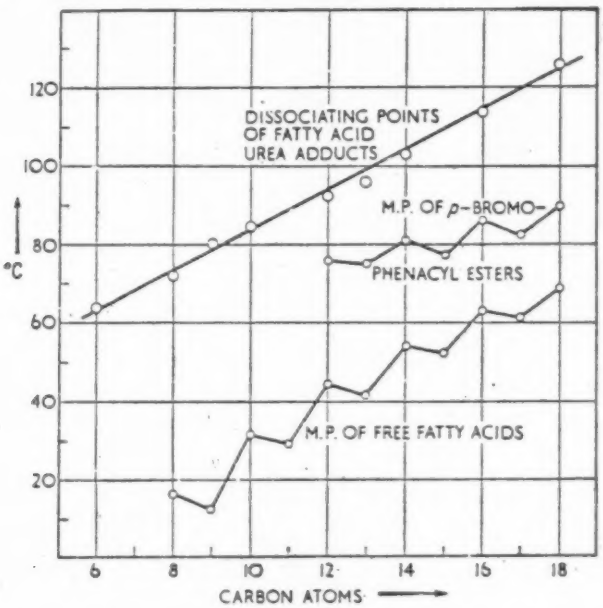


Fig. 9. Temperatures of dissociation of saturated fatty acid urea adducts, melting points of free acids and of p-bromophenacyl esters.

are singled out in the urea lattice. SWERN and co-workers also report the characterization of alcohols and the method certainly is not limited to the groups so far tested.

Preservation against oxygen

Another remarkable property of the urea inclusion compounds must be traced back to their particular crystalline state. H. SCHLENK and HOLMAN observed tnat unsaturated fatty acids bound in urea are perfectly stable against autoxidation. This stability became apparent when the urea compounds of unsaturated fatty acids did not develop rancidity. The stability has been demonstrated conclusively by following the peroxide values of both free and bound soybean fatty acids over a period of several weeks.[39] The oxygen uptake of pure linoleic and linolenic acids and of their adducts also has been measured.[40] (Fig. 10.)

The autoxidation of unsaturated fatty acids is known to be a chain reaction.

Because the contact of donor and acceptor molecules is prevented in the crystals, the chain mechanism cannot operate. Furthermore the urea probably offers a barrier against the free penetration of oxygen.

The necessary precautions against autoxidation are greatly reduced by this stabilization in all urea fractionations. The adducts are an ideal storage form, at least for laboratory scale amounts. They have been used directly for the preparation of feed rations in nutritional studies on essential fatty acids where the presence of urea did not interfere.[17] This application is limited to free acids having less than four double bonds, for ABU-NASR, POTTS, and HOLMAN

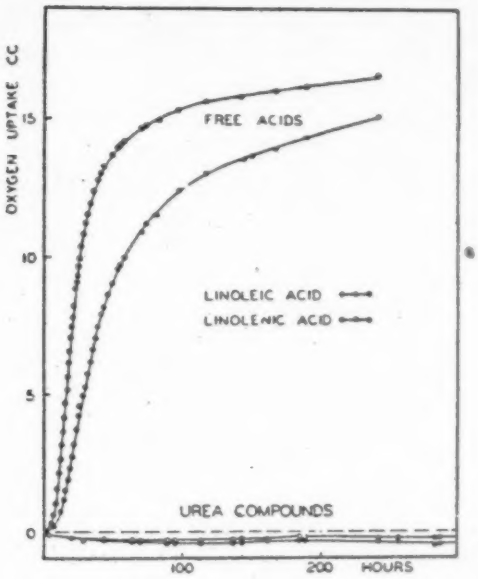


Fig. 10. The oxygen absorption of linoleic and linolenic acids and their urea compounds at 37° under oxygen.
(from H. SCHLENK and HOLMAN[68]).

encountered difficulties in binding highly unsaturated fractions of cod-liver fatty acids. When esterified, however, the same acids react readily with urea.[27]

FRACTIONATIONS BY MEANS OF UREA

Straight chains from branched compounds

The principle of such separation is to bind all potential adduct formers and to leave the heavily branched components unchanged. The method is of interest for the removal of free acids from triglycerides. RIBAS and GIL refined fish oils by heating them with urea, diluting the reacted mixture with benzene and centrifuging off the solids.[31] The acid numbers of the original material and of the resulting fractions were compared on the basis of free C_{18} acids. A content of 10% free acids in sardine oil was reduced to 3·6% in the residual oil, whereas the fraction isolated from urea contained 51% acids. From 26% acids in cod-liver oil, 14% were retained by the glycerides after one treatment with urea, and

6·9% after a second treatment. No substantial change was achieved when the oil contained only 5% free acids. Urea precipitation of acids from various other triglycerides has been mentioned by MEADE,[25] MASELLI and NORD,[23] and by MORENO, RONCERO, and JIMENES.[26] The latter authors also treated turpentine resin acids containing fatty acids. The procedure should apply equally to the otherwise rather cumbersome separation of mono-, di-, and triglycerides, for which preliminary experiments with urea have been reported to give unsatisfactory and unexpected results.[33]

The constituents of wool wax, i.e. fatty acids, higher aliphatic alcohols, and sterols, were separated by saponification and subsequent binding of the straight chain alcohols. The fatty acids had been eliminated by the usual alkaline extraction of the saponification mixture. From 100 g of unsaponifiables, 21 g of straight chain alcohols were obtained by precipitation with urea in ethanol and recrystallization of the adduct from the same solvent. The sterols remained in the mother liquor (v. RUDOLFF[34]). Unsaponified sperm oil has been fractionated by MEADE[25] (see p. 254).

In a study on fatty acid vinyl esters, SWERN and PORT made use of the urea method for purifying vinyl pelargonate from cross-linked contaminants and also for recovering monomer vinyl palmitate from partly polymerized material.[53]

Fractionation of various straight chain compounds

The influence of characteristic groups upon urea addition has already been discussed in the theoretical outline. Tests using two-component blends showed that an effective segregation of long and short chain fatty acids and also of saturated and unsaturated acids can be achieved by one urea precipitation.[40] The main interest has been focused on the fractionation according to unsaturation. The protection against oxygen and the convenient temperature offer advantages over the conventional low temperature crystallization, although the amount of urea carried with the fatty acids is often an impediment. Moreover, recovery of the free material requires an additional step. In the series palmitic or stearic, oleic, linoleic, and linolenic acid the efficiency of separation is best between oleic and linoleic acid. The importance of this fact is illustrated by the following example: Soybean fatty acids, I.N. 132, were fractionated by urea precipitation into two approximately equal parts having I.N. 77 and 169. Oleic and linoleic acid, the major components of this oil, had been enriched and were the main constituents in the respective fractions. The linoleic acid fraction was reconverted into glycerides and proved to have drying properties equal to linseed oil.[28] Such segregations have been carried out with a large variety of fats. In the experiments listed in Table 7, the amount of the more saturated, precipitated fraction is regulated by the amount of urea,[39], [40] except in the fractionation of rice oil, where the amount of methanol had also been varied systematically.[35] Similarly, cotton-seed fatty acids have been separated into four fractions having I.N. 18, 115, 187, and 193 (filtrate). Besides fractionation, a purification of the material was achieved since the colour of the prepared acids was improved and the contaminants were concentrated in the filtrate.[7a]

Table 7. Enrichment of saturated and unsaturated fatty acids

	Acids		Urea	Methanol	Acids			
					In urea		In filtrate	
	g	I.N.	g	ml	g	I.N.	g	I.N.
Chinese tallow	51	19	100		27	6·5	18·5	38
Olive oil	50	81	50		13	46	37·7	85·5
Corn oil	50	126·5	75		21	81	26	164
Gourd seed oil	49	148	50		14·7	81	34	160
Soybean oil	100	141	30		9	56	81	162
Soybean oil	100	141	100		27	88	56	180
Soybean oil	100	141	200		67	119	27	191
Linseed oil	50	169	75		20	109	29	204
Rice oil	20	99	20	120	4·2	29	15·1	115·9
Rice oil	20	99	40	240	7·3	40	11·9	134·8
Rice oil	20	99	60	360	10·8	51	8·4	159

The formation of inclusion compounds with insufficient amounts of urea is competitive for the various components of a mixture and the composition of the solids should largely follow the individual equilibrium constants. The "trailing in" effect, which has been discussed on page 246 for branched compounds, certainly applies here too and counteracts a clean-cut separation of straight-chain components. The different equilibrium constants influence each other unfavourably for separation.

SWERN and PARKER tested the efficiency of the method for the specific problem of preparing oleic acid from various commercial sources.[50] Their objective was to apply the urea separation to fats of low quality, comparable with crude industrial materials. In their procedure the bulk of saturated acids was removed by crystallization from 90% methanol at 0°. Urea was then added to the filtrate and the adduct crystallized at room temperature (Table 8).

The authors discuss the merits and disadvantages of the urea procedure and of solvent preparation of oleic acid. In addition to the general considerations given above, they emphasize for this special case the improved colour in favour of the urea method. On the other hand, the yields are somewhat lower and urea is less efficient in removing polyunsaturates than low temperature crystallization. Intentionally, the recovery of urea and of methanol has not been considered. SWERN and PARKER also studied the use of urea in concentrating linoleic and linolenic acid. They obtained 85–95% pure linoleic acid from corn oil, the recovery being 72—50%, and 87—89% pure linolenic acid from perilla oil, recovery 61—55%. The technique was not so efficient in concentrating linolenic acid from linseed oil fatty acids. There the practicable enrichment apparently reaches a limit with 70% purity, the recovery of linolenic acid, however, being excellent. A levelling off in the efficiency of separating the higher unsaturated acid is clearly indicated in the experiments.[52]

In an attempt to isolate fatty acids containing four, five, and six double bonds, ABU-NASR, HOLMAN, and POTTS concentrated the highest unsaturated acids of

Table 8. Enrichment of oleic acid by fractionation of industrial fatty acids

Fatty acids*	g	Acids from solvent cryst.		Acids from urea precipitate*				Acids from filtrate	
				Total		Oleic acid			
		g	I.N.	g	I.N.	Recovery %	Purity %	g	I.N.
Artificial blend:									
Myristic 0·6 .									
Palmitic 6·0 .	9·6	8·4	1·7	1·6				0·5	
Stearic 3·0 .					73	70	78		121
Oleic . . .	9·0			6·4				1·3	
Linoleic . . .	1·4			0·16				1·6	
Prime tallow:									
Saturated . .	407	429	4·2	74					
Oleic . . .	532			412	79	77	83	87	131
Polyunsatd. . .	61			10					
Brown grease:									
Saturated . .	480	543		(§) 99	(§)				
Oleic . . .	481			236	65·3	49	69	94	105
Polyunsatd. . .	39			6·8					
Inedible tallow:									
Saturated .		325	10·2	(‖) 4·4	(‖)				
Oleic . .	1000			138	89·7	26	95	38	127
Polyunsatd. .				2·9					
Red oil:†									
Saturated . .	150			(§) 88	(§)				
Oleic . . .	700	(¶)	(¶)	528	79·2	76	84	222	132
Polyunsatd. . .	150			12·6					
Oleic acid:‡									
Saturated . .	150			103					
Oleic . . .	700	(¶)	(¶)	616	79·8	88	84	244	136
Polyunsatd. . .	150			14·7					

* Assay of components see BRICE *et al.*[9]
† Single-distilled commercial oleic acid.
‡ Above commercial material, straight run redistilled.
§ After straight run distillation.
‖ After fractional distillation.
¶ Solvent crystallization omitted.

marine fish oil in successive steps. The mixture of maximum unsaturation precipitated by urea had an I.N. of 215, whereas the mixture left behind, I.N. 350, did not react upon further addition of urea. The methyl esters of the same material, however, were bound up to I.N. 346, and the filtrate had I.N. 344. The results were almost equal for methyl, ethyl, propyl, and butyl esters. In their interpretation, the authors accordingly give more weight to the different character of the carboxyl and ester groups than to the increase in chain length. From esters of cod liver, shark liver, pollack liver, menhaden, salmon and herring

oil, unsaturated concentrates were obtained having I.N. between 294 and 356.[27] Subsequent displacement chromatography showed that these were still mixtures of fractions having I.N. between 280 and 450.

Preparation of pure unsaturated acids and esters

The preliminary solvent crystallization in the above preparations of oleic acid concentrates serves mainly to eliminate saturated acids which would be precipitated with the bulk of oleic acid in adduct form. This is done not only to restrict the urea as closely as possible to the amount necessary for binding the desired component, but also to compensate for the limitations of the urea method, which is not practical for separating the saturated acids from oleic acid. Since in natural mixtures different degrees of unsaturation as well as various chain lengths are represented, there will be always an enrichment of C_{12}—C_{16} acids along with the unsaturated fractions of higher acids. The tendency to form the urea adduct may be estimated to be about equal for oleic acid and a saturated C_{18} acid. However, the efficiencies of distillation and urea precipitation supplement each other effectively in regard to the two variables. Distillation allows separation according to chain length so that in the fractions of a distilled product this factor cannot interfere any more with the urea separation according to unsaturation. H. SCHLENK and HOLMAN prepared methyl oleate and methyl linoleate by a combination of both methods.[40] In applying an additional low temperature crystallization, SWERN and PARKER obtained better yields of oleic acid and ester with a lower content of polyunsaturates.[51] (Table 9.)

Table 9. Preparation of pure unsaturated acids and esters

Material used	*g*	*I.N.*	*Compound obtained*	*g*	*I.N.*	*Quality*
Olive oil,[40] methyl esters	463	84	Methyl oleate .	184	88·5	2–3% linoleate, no saturates
Olive oil[51] . . .	1000	78	Methyl oleate .	540	84·9	0·2% linoleate 0·9% saturates
Olive oil,[51] acids . .	1000	81·3	Oleic acid .	500	88	0·1% linoleic acid 2·2% saturates
Corn oil,[40] methyl esters.	200	125	Methyl linoleate	28	173	Theoretical I.N. 172·5
Corn oil,[40] methyl esters.	200	125	Methyl linoleate	46	168	Theoretical I.N. 172·5

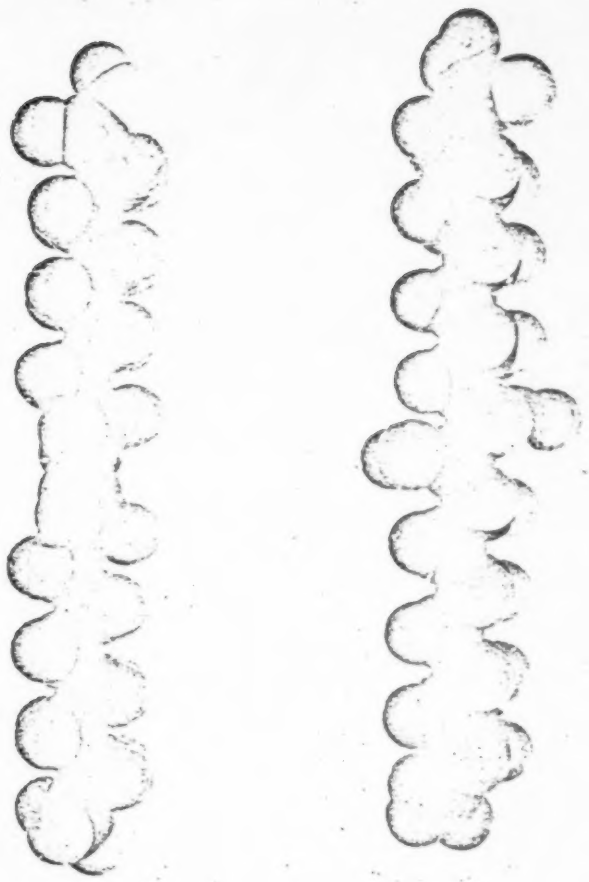
Separation of modified materials

It is obvious from the theory of adduct formation that the procedures of segregation described above are applicable to mixtures of fatty acid nitrils, of fatty alcohols,[28] or others without markedly changing the pattern. In special cases, however, the efficiency of fatty acid separation can be improved by altering some of the constituents of a mixture into derivatives for which a pronounced change in their reaction with urea is to be expected. This has been carried out experimentally by MEADE in a separation of ricinoleate from other castor oil

esters lacking an alcoholic group. The methyl esters were heated with boric acid to esterify the hydroxyl containing moiety. The orthoborates are heavily branched and the type of separation resembles the removal of acids from triglycerides, except that water and alcohols must be rigorously excluded since they would split the boric acid esters. Acetone was chosen as the solvent and after hydrolysis of the portion not bound by urea, 80 g of pure methyl ricinoleate was obtained from 100 g castor-oil esters. Although acetylation produces the same effect as this esterification, the quality of the product obtained from commercial acetylated castor-oil esters was not quite as good.[25]

Another accessory reaction is the bromination of double bonds, for the rather bulky bromine inhibits adduct formation when it is not in the ω-position. It has thus been possible to isolate the methyl esters of unsaturated C_{16} acids in brominated form from parsley seed oil. Petroselinic acid was removed from the acids by solvent crystallization. After esterification a close C_{16} fraction was distilled using methyl palmitate as a carrier. The greater part of the palmitate could be eliminated by crystallization and the residue containing the unsaturated C_{16} methyl esters was brominated. From the mixture of bromo-esters and methyl palmitate, only the latter reacted with urea. The bromo-esters were isolated from the mother liquor and treated with zinc to yield hexadecenoic ester (0·2%) and hexadecadienoic ester (0·05%).[25] Unfortunately, no procedure is known for direct dehalogenations which can claim to render the genuine unsaturated material.

Without discussing the detailed structure of peroxide intermediates of fatty acid autoxidation, one may expect that the presence of a peroxide group will change greatly the readiness of unsaturated fatty acids to combine with urea. This has been established by treating autoxidized soybean fatty acids with urea, whereby the different fractions were either depleted of peroxides or enriched without appreciable decomposition.[39] Coleman, Knight, and Swern developed the urea technique for obtaining highly concentrated methyl oleate peroxides.[5] Since secondary products become prevalent after a maximum content of peroxides has been reached in the course of autoxidation, it is necessary to process the material before the peak level of the peroxide number has been passed. Otherwise the separation of the oleate peroxides from the mixture is unsatisfactory and an unnecessary amount of methyl oleate is lost. In one of the procedures described, 350 g of autoxidized methyl oleate containing 15·2% peroxides was added to the stirred boiling solution of 1500 g urea in 3 litres of methanol. Without further heating, stirring was continued and the precipitate filtered at room temperature. The peroxides were isolated from the filtrate as 44 g of a pale yellow oil containing 83% peroxides. Recoveries of 60–75% of the initial peroxides in 80–90% concentrations are thus reproducible. The methyl oleate precipitated by urea is essentially free of peroxides and can be re-used after distillation. It was impossible to obtain the peroxides in a purity above 90%. As shown in other investigations by Swern and co-workers, 9.10-dihydroxystearic acid, m.p. 131°, does not form an adduct with urea, although it may be "trailed in" by adduct formers.[54] Experiments using higher autoxidized



a *b*

Fig. 11*a*. Oleic acid. Fig. 11*b*. 9,10-dihydroxystearic acid, m.p.-131°. The straight molecule and the regular zigzag constellation are re-established after cis-hydroxylation in turning the C_1–C_8 portion by 180°. The hydroxy groups are in the widest possible distance and the molecule is slightly turned to bring this diameter into the plane of projection.

(To face page 262)

methyl oleate suggest that this is one of the contaminants present in the concentrate which escape precipitation even when the urea treatment is repeated. The method proved to be useful also in concentrating peroxides of methyl elaidate, linoleate and linolenate. It must be mentioned that CATRAVAS and KNAFO found urea not to be selective in the fractionation of linseed oil fatty acids when autoxidized at 100°.[4] Methyl oleate and linoleate, when autoxidized below 30° in ultraviolet light gave results which conform better with the above findings.[4a] [19] Similar experiments have been carried out with autoxidized acids of castor oil.[4b]

In Table 10 an attempt has been made to compile the applications of the urea reactions reported for natural mixtures of fatty acids.

Table 10. Natural mixtures treated with urea

Material	*Purpose of treatment*
Castor oil	Preparation of methyl ricinoleate[22] segregation of autoxidation products[4b]
Chinese tallow	Segregation of saturated and unsaturated[40]
Corn oil	Segregation of saturated and unsaturated.[7a], [23], [40] Preparation of methyl linoleate[40]
Gourd seed oil	Segregation of saturated and unsaturated[40]
Linseed oil	Segregation of saturated and unsaturated[23], [28], [40], [52]
Olive oil	Segregation of saturated and unsaturated[40]. Preparation of methyl oleate,[40] of oleic acid[51]
Parsley seed oil	Preparation of hexadecenoic acid[25]
Perilla oil	Segregation of saturated and unsaturated[52]
Rape seed oil	Preparation of erucic acid[19], [45a]
Rice oil	Segregation of saturated and unsaturated[35], [36]
Soybean oil	Segregation of saturated and unsaturated[28], stabilization, segregation according to peroxide value[39], [40]
Sunflower oil	Preparation of linoleic acid dihydrobromide[25]
Fat of *Fusarium lini* Bolley	Isolation of stearic acid[23]
Cod liver oil	Enrichment of highest unsaturates,[27] deacidification[31]
Herring oil	Enrichment of highest unsaturates[27]
Menhaden oil	Enrichment of highest unsaturates[27]
Pollack liver oil	Enrichment of highest unsaturates[27]
Salmon oil	Enrichment of highest unsaturates[27]
Shark liver oil	Enrichment of highest unsaturates[27]
Sardine oil	Deacidification[31]
Sperm oil	Isolation of wax esters[25]
Wool wax	Characterization of acid fraction,[55] isolation of wax alcohols[34]

UREA ADDITION AS AN AID IN DETERMINING STRUCTURES

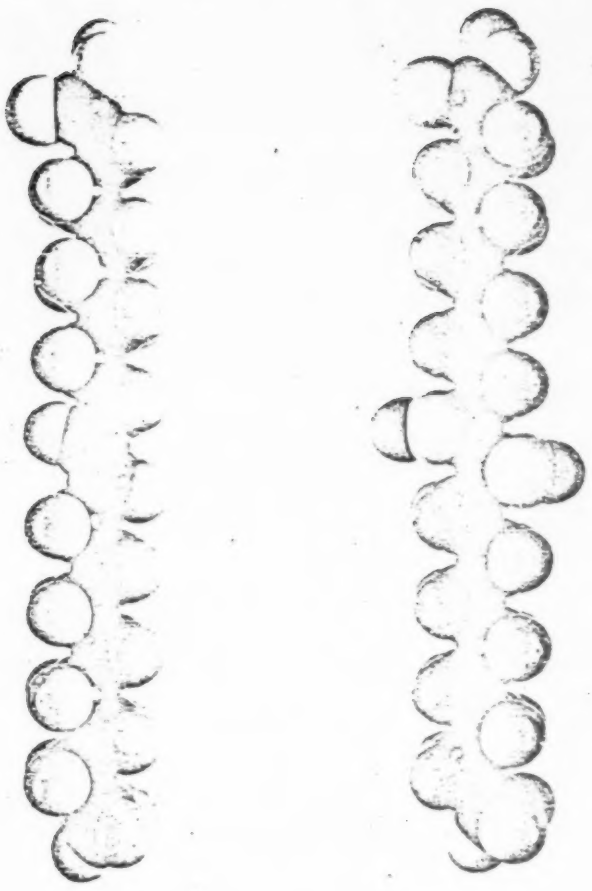
In the theoretical section it has been pointed out that adduct formation is conditionally related to the diameter of the reacting compound. For a rather large group of structures one cannot safely predict whether they will be taken up by hexagonal urea. Several interesting attempts, however, have been made to use urea as a test reagent for deciding between alternative structures.

It is generally accepted that hydroxylation of double bonds with alkaline potassium permanganate results in *cis* addition.[11], [18], [57] This result has also been assumed for the oxidation of oleic acid which yields a 9,10-dihydroxystearic acid, m.p. 131°, and for the same oxidation of elaidic acid, yielding an isomeric 9,10-dihydroxystearic acid, m.p. 96°. The relations are reverse when the two isomers are prepared via the epoxides of oleic and elaidic acid by oxidation with organic per-acids and subsequent hydrolysis of the oxirane ring. Reactions implying chlorohydroxystearic acids as an intermediate render the picture still more complex.[49] It was desirable to find further support for the above surmise of normal permanganate oxidation of oleic and elaidic acid.

SWERN, WITNAUER, and KNIGHT calculated the diameters of the two, 9,10-dihydroxystearic acids which would result from *cis* addition, under the assumption that the normal zigzag structure for both hydroxylated acids is maintained.[54] Figs. 11 and 12 show the positions which the hydroxy groups probably occupy. The diameter is approximately 6·0 Å for the glycol m.p. 131°, originating from the *cis* double bond (oleic acid); for the glycol m.p. 95°, originating from the *trans* double bond (elaidic acid) they find 5·4 Å (PAULING's values[29] have been used). The two diameters, 5·4 and 6·0 Å, are close to the limit capacity of the lumen in hexagonal urea and their difference should be reflected in the urea reaction. In the tests 5 g of acid and 25 g of urea were dissolved in 100 ml of methanol. From 9,10-dihydroxystearic acid, m.p. 95°, a urea compound was obtained in good yield, whereas the isomer, m.p. 131°, crystallized without urea at room temperature. When a 1 : 1 mixture of the acids was reacted the urea precipitate contained the isomers in a ratio 3 : 1. Both methyl esters formed urea compounds, a result which the authors attribute partly to the different status of hydrogen bonding in free and esterified carboxy groups, and partly to the increase in chain length. The temperatures of dissociation can be taken as a relative measure of stability. The ester offering greater obstruction to inclusion, that derived from oleic acid, dissociates at 114° and the adduct of the isomer has a dissociation point of 120°. All these observations strongly support the initial assumption that the permanganate oxidation of the octadecenoic acids proceeds as the usual *cis* addition.

Two formulas are discussed in the recent literature for phellonic acid, one of the constituents of the acids found in cork. The alternatives are either the structure of 22-hydroxydocosanoic acid[15] or of 22-hydroxytetracosanoic acid.[7] RIBAS and CURBERA found that the acid, as well as the acetylated acid, easily reacts with urea. This result is ambiguous since TRUTER[56] found that an ethyl side chain can be included. The authors postulate phellonic acid to be 22-hydroxybehenic acid.[32] The same authors prepared urea compounds from several other cork acids and report abnormal composition for the adducts of phloionic (9,10-dihydroxyoctadecanedioic) acid and phloionolic (9,10,18-trihydroxyoctadecanoic) acid.[31] The weight ratios of urea : acid are 0·2 : 1 and 0·24 : 1 in these compounds instead of the normal value close to 3 : 1. They obviously do not belong to the group of inclusion compounds discussed here.

In studying triple bond fatty acids MEADE isolated bolekic acid from boleko



a *b*

Fig. 12*a*. Elaidic acid. Fig. 12*b*. 9.10-Dihydroxystearic acid, m.p. 95°. The straight molecule and the regularity are maintained by a slight turn of the C_1–C_8 portion.

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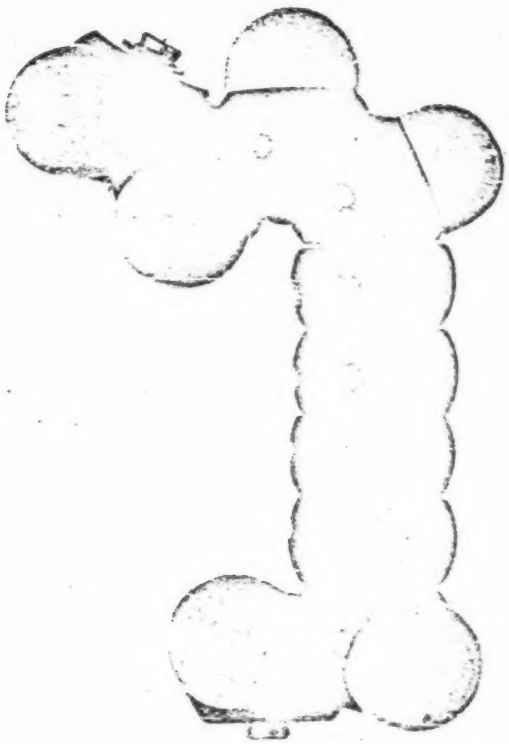

Fig. 13. The unsaturated portion of bolekic acid. The conjugation of a cis double bond with acetylene bonds causes great irregularity in the molecule.

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oil. This C_{18} acid contains an ethylene bond apparently in conjugation with two acetylenic bonds, the exact position of the established fragment

$$—C{\equiv}C—C{\equiv}C—C{=}C—$$

not being known as yet. In the test with urea, bolekic acid does not form an adduct.[25] Particularly a *cis* double bond, conjugated with triple bonds as MEADE suggests for the structure, will inhibit inclusion of the molecule (Fig. 13). Since nothing has been reported so far on the addition of acetylene compounds to urea, the influence of the triple bonds on the reaction cannot be evaluated.

Although the reaction of urea with fatty acids has been studied extensively only in the last few years, one may well affirm that the fundamentals are established. Although the preparative use of urea is very recent in comparison with the standard procedures as distillation or low temperature crystallization, the new method is already recognized as an important tool. For industrial applications only a partial replacement of former methods may be expected. Information on this is rather scarce. Some of the findings are challenging for such development in spite of the obvious difficulties for industrial use of the urea reaction. In research laboratories concerned with special problems of fatty acid chemistry, urea is being widely used and its application is certain to be extended.

Finally, it must be mentioned that urea is not the only substance which forms inclusion compounds with fatty acids or their derivatives. The choleic acids, adducts of desoxycholic acid and fatty acids[48], [58] were among the first purely organic compounds which are now recognized as belonging to the class of inclusion compounds.[13], [14], [43] Recently FREUDENBERG and CRAMER found that cycloglucosanes form adducts, particularly with lipophilic substances.[6], [12] With urea the principles governing inclusion compound formation have been laid down and most likely the urea adducts will be the model for further types to be discovered.

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6

INFRARED ABSORPTION SPECTROSCOPY IN FATS AND OILS

D. H. Wheeler

Introduction

The infrared region is shown in its relationship to the general electromagnetic spectrum in Fig. 1. The ultraviolet, visible and infrared regions are those which have been developed most extensively for qualitative and quantitative analysis in organic chemistry by the use of absorption spectroscopy, the measurement of wavelengths at which compounds absorb electromagnetic radiations.

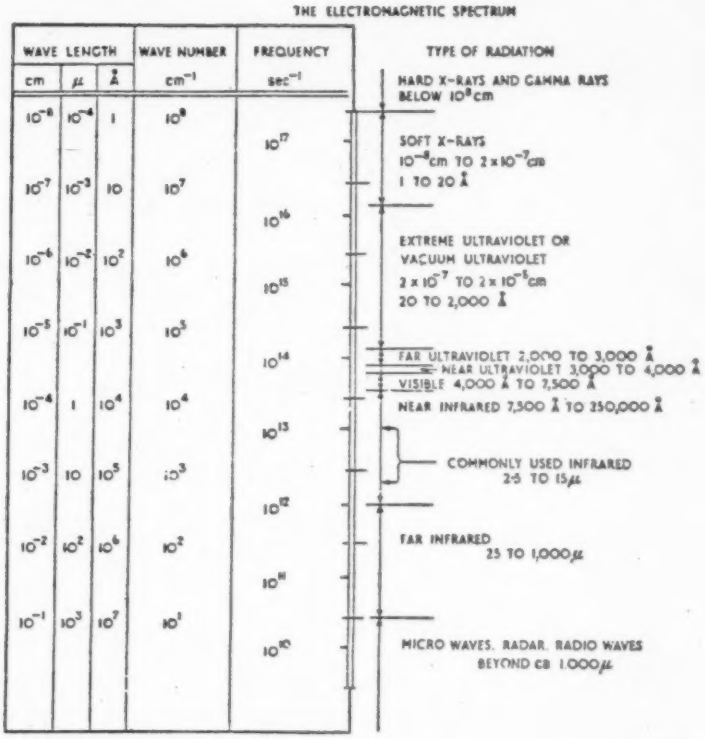
The ultraviolet and visible absorption of compounds is largely related to the frequencies of oscillation of the outer electrons of their atoms. This field of absorption spectroscopy has been extensively developed over a number of years. It is particularly useful in studying compounds containing unsaturated groups where the electron frequencies correspond to visible and ultraviolet wavelengths which can be most conveniently measured. Whereas non-conjugated carbon to carbon double bonds absorb in the extreme or vacuum ultraviolet (below 2000 Å), conjugated double bond systems absorb at wavelengths above 2000 Å, in the range where readily available instruments can measure absorption. The use of ultraviolet absorption for detecting and determining conjugation in fats and oils is well established.

The infrared absorption of compounds is largely associated with the frequency of vibration of the atoms in the molecules (near infrared) and the frequency of molecular rotation (far infrared). Infrared absorption spectroscopy is a field of relatively recent and rapid development. It is valuable for studying structures and for characterizing a wide variety of compounds. It promises to become more useful as correlations are further developed between specific structures and the wavelengths and nature of the corresponding absorption bands.

Microwave absorption of compounds is largely associated with molecular rotation frequencies. This is a very new and relatively unexplored field which may prove quite valuable as methods of measurement are developed.

While the infrared portion of the spectrum includes the region from about 0·75 to 1000μ, the section from 2·5 to 15μ has been the most thoroughly studied. This is largely because of fewer limitations in sources of radiation and prism materials, which has permitted the construction of satisfactory spectrophotometers covering this range. The present discussion will be confined to this commonly used region. Fortunately, this section from 2·5 to 15μ is perhaps the most valuable part of the infrared spectrum, since it covers most of the region of fundamental molecular or interatomic vibrations (2·5 to 25μ).

These interatomic vibrations occur with frequencies which are affected by the masses of the atoms and the strength of the bonds between them just as the frequency of vibration of a weight suspended on a coil spring will depend on the mass of the weight and the stiffness of the spring. If a certain interatomic bond, such as C—H, causes definite frequencies of interatomic bending or stretching, one may expect absorption of infrared radiation at wavelength corresponding to these frequencies of vibration. These frequencies have been calculated from theoretical considerations for certain molecules (mostly simple or symmetrical),



and the observed frequencies or wavelengths of absorption have agreed well with the calculated values. However, most of the assignments of wavelengths of absorption bands to specific interatomic bonds or structures has been made by the empirical method of comparing the infrared spectra of a sufficient number of compounds having a certain structure in common. By this procedure, many types of chemical bonds have been characterized by definite wavelengths or regions of infrared absorption. Adjacent structures may affect the intensity and wavelength of their infrared bands. The effect of such interactions is becoming better known as more compounds are studied in detail.

In addition to the absorption bands which can be assigned with some certainty to definite bond structures, many other bands are usually observed which are characteristic of the whole molecule, perhaps because of interactions not

known at present, or because of overtones (multiples of fundamental frequencies) or combination bands (sums or differences of fundamentals). The whole spectrum of these bands is so characteristic of over-all chemical structure that the infrared absorption spectrum of a compound is a highly characteristic property, and permits determination of identity or non-identity of materials when other methods may fail. It has been called the "fingerprint" of the molecule.

Units of measurement

The micron, μ, is the commonly used unit of wavelength in infrared measurements. For uniformity, it will be used throughout.

$$1 \text{ micron} = 10^{-4} \text{ cm} = 10^{-3} \text{ mm} = 10{,}000 \text{ Å} = 1000 \text{ m}\mu$$

Frequencies are also often used to measure infrared bands. Frequency is a more fundamental unit, in that it is independent of the medium; and theoretical calculations of vibrations related to infrared absorption are directly calculated as frequencies, rather than wavelengths. Frequencies are usually expressed as reciprocal centimetres, cm^{-1} or wave number, which is the number of waves per centimetre of path in vacuum.

$$\text{Frequency, cm}^{-1} = \frac{10{,}000}{\mu}$$

Frequency as vibrations per second is sometimes used:

$$\text{Frequency, sec.}^{-1} = \frac{\text{velocity of light}}{\text{wavelength}}$$

Instruments

The essential parts of an infrared spectrometer are: (1) A source of infrared radiation such as an incandescent filament of rare earth oxides (Nernst Glower), or of carborundum (Globar). (2) An optical dispersion system to disperse the radiation enough so that bands of very narrow wavelength can be isolated by a narrow slit. Prisms made of sodium chloride, lithium fluoride, potassium bromide and calcium fluoride are used. Rock salt prisms are the most commonly used, since they are quite transparent from 2·5 to 15μ, and give sufficient dispersion for many purposes. Below 5μ, rock salt gives rather poor dispersion. Therefore, prisms made from lithium fluoride or calcium fluoride are more suitable if high resolution is desired below 5μ. Gratings are used less frequently. (3) A sensitive means of detecting, amplifying and measuring the intensity of the narrow band of radiation which falls on the slit. A thermocouple or a bolometer may be used as detector. In order to be able to amplify the small direct current voltage generated by the detector, it may be converted to a pulsating or alternating current by a rotating sector in the optical path, or by a commutator in the electrical circuit. The pulsating current may be amplified by vacuum tube amplifiers and measured or recorded. (4) A means of placing the sample in the

path of the radiation. A cell containing the sample is usually placed between the source and the optical system. Cell windows are commonly made of polished rock salt, since it is sufficiently transparent in the 2·5 to 15μ range. Cells are usually 10 to 30 cm long for gases, and 0·01 to 1 cm for liquids. Solids may be examined in solution, as a thin sheet or plate, or as a fine suspension ("mull," frequently in Nujol or a perfluorinated hydrocarbon to give transparency in the C—H region).

Complete infrared spectrophotometers are available from *National Technical Laboratories* (Beckman), *Perkin-Elmer Corp.*, *Baird Associates*, *Unicam Instruments*, *Hilger*, and *Sir Howard Grub, Parsons and Co.* The more advanced models from each of these concerns are equipped with automatic recording devices which record per cent transmission directly against a standard chosen as 100% transmission (empty cell, cell window plate, air, or cell filled with solvent if solution is used). Such an instrument saves much labour and time compared to manual instruments, where successive values must be read individually, calculated and plotted to obtain a complete infrared curve. The double beam instruments (*Baird* and *Perkin-Elmer*) use a reference beam which is balanced against the beam passed through the sample so as to read per cent transmission directly on an indicating and recording instrument. A single beam instrument (Beckman) records the signal from the 100% standard on a magnetic wire or tape, and then feeds this signal back into the machine during the actual run on the sample so as to give direct per cent transmission readings on the recording instrument.

General Correlations of Structures and Absorption

Any general discussion of correlation of structures with absorption bands must be made with considerable caution, realizing that many such correlations are empirical. Some are based on a large number of compounds and are well established. Others are based on more limited or less certain data, and are tentative or speculative.

Some of the more important assignments of wavelength related to the structure of fatty acids and their derivatives will be discussed.

Carbon-hydrogen bonds

C—H stretching bands of —CH_2— occur at 3·42μ and 3·51μ. These two bands are quite strong in fatty acids and derivatives because of the large number of CH_2 groups present.

C—H stretching bands of —CH_3 occur at 3·38μ and 3·48μ. In fatty acids and esters, these bands are relatively weak, and require good resolution to detect them in the presence of the stronger adjacent —CH_2— bands.[1]

A band at 3·31μ is due to C—H stretching associated with the C=C double bond. This has been assigned to the C—H bond of the double bonded carbon by some workers[1] while others assign it to the C—H bond of a methylene adjacent to a double bond.[2] This band is usually not readily observed with the rock salt prism because of its proximity to the strong CH_2 bands at 3·42 and 3·51μ.

Methylene groups also show bands at 6·84μ (bending) and 13·90μ (rocking). Several groups between 6·85 and 7·15μ have been ascribed to —CH_2— bending frequencies in fatty acids.[1]

The C—H stretching band of aldehydes occurs at 3·6 to 3·7μ.

The C—H stretching band of terminal acetylenes occurs at about 3·0μ.

The C—H bending band of non-conjugated *trans* double bonds occurs at 10·3μ. This is a strong band, valuable in studying *cis-trans* isomerism of unsaturated acids. Non-conjugated *cis* double bonds absorb at about 14μ, but this band is obscured by a band due to multiple CH_2 groups in fatty acids and esters.

The C—H bending band of *trans, trans* conjugated dienes occurs at 10·12μ, while *cis, trans* conjugated dienes show a doublet at 10·18 and 10·54μ.

Terminal double bonds show bands at about 10·0 and 11·0μ.

Carbonyl frequencies, C=O stretching bands

These bands are usually quite strong. The various types of C=O groupings fall in the general range from 5·5 to 6·5μ. The wavelengths listed below are subject to some variations in different structures. Conjugation usually shifts the bands to longer wavelengths by 0·05 to 0·01μ.

Structure	*C=O band wavelength μ*
Acid	5·85
Ester	5·75
Anhydride	5·48 and 5·63
Amide, unsubs.	5·9
Amide, monosubs.	5·9
Amide, disubs.	6·0
Aldehydes	5·8
Ketones	5·8

Carbon-carbon double bond stretching

The C=C stretching band occurs at 6·03μ. It is a rather weak band, and is obscured by its proximity to the strong acid or ester C=O. When the double bond is near the middle of the chain, as in oleic and elaidic acids, it is absent from the *trans* isomer, due to symmetry.

Carbon-oxygen bonds

Bands at 8·0, 8·4, and 8·5μ have been ascribed to ester C—O stretching in methyl esters.

Hydroxyl bending absorption occurs at 9·5μ in the fatty alcohols.

The oxirane or epoxy structure shows bands at 11 and 12μ for terminal groups. Epoxides made from *cis* mono-unsaturated fatty acid compounds show a band at about 11·9μ, while those made from the *trans* isomers show at about 11·3μ.

Oxygen-hydrogen bonds

Free O—H stretching band occurs at 2·7 to 2·8μ, whereas associated O—H— stretching shows at 3·0 to 3·3μ. The hydroperoxide group shows a very similar band. The OH group of free acid COOH is fairly broad and weak, compared to the strong, sharp methylene bands at 3·4 and 3·5μ, and is not easily observed in fatty acids.

C=N of Nitriles

The C=N stretching band is a weak but sharp band occurring at 4·45μ.

A general chart of correlations of structure with wavelength is shown in Fig. 2. Various texts also give tables of such correlations.[3], [4], [5], [6]

Recent Applications of Infrared to Fats, Fatty Acids and Derivatives

Saturated and mono-unsaturated fatty acids and esters

Shreve, Heether, Knight, and Swern[7] reported infrared absorption of a number of saturated and mono-unsaturated fatty acids, methyl esters, triglycerides and alcohols. Bands common to all the compounds were:

1. A band near 3·4μ, due to C—H stretching (plus bonded O—H——O in the acids).
2. A singlet at 6·9μ or doublet at 6·9 and 7·0μ due to C—H bending.
3. A band at 7·2–7·4μ due to CH_3 (absent in undecylenic acid).
4. A band near 13·9 due to chain of 4 or more CH_2 (plus *cis* C=C bending in *cis*-unsaturated acids).

The fatty acids showed a band at 3·7μ which was due to bonded OH——O which fused with the 3·4μ band due to C—H stretching. They also showed a broad band at 10·7μ due to vibration of the OH of the COOH group, a doublet at 7·8 and 8·0μ due to C—O— vibration of the COOH group, and a band at 5·85μ due to C=O stretching of the COOH group.

The methyl esters did not show the —OH bands at 3·7μ or 10·7μ found in the acids. The C=O stretching band occurred at 5·75μ, and a triplet at 8·0, 8·3, and 8·5μ was observed for the C—O— vibrations.

The triglycerides showed the same C=O frequency 5·73μ as the methyl esters. The C—O— bands were at 8·6μ (stronger) and at 8 and 9μ (weaker).

The alcohols showed a band at 9·5μ due to —OH bending, and a band at 2·94 due to O—H stretching (2·7μ in solution).

Trans mono-unsaturated compounds showed the band at 10·36 due to =C—H out of plane vibration. *Cis*-mono-unsaturated compounds contributed to the 13·9μ band due to chain of four CH_2's. Terminal unsaturation (undecylenic) showed bands at 10·0 and 11·0. A band at 6μ due to *cis* C=C was barely observable on the side of the C=O band at 5·75–5·85μ.

O'Connor, Field, and Singleton[8] reported infrared spectra of the even carbon saturated acids and their methyl and ethyl esters from C_6 to C_{18}. They reported extinction coefficients at the significant wavelengths at various

concentrations. They observed that a band at 9·6μ distinguished ethyl esters from methyl esters.

Detection and determination of isolated trans double bonds

McCUTCHEON, CRAWFORD, and WELCH[9] examined the infrared and Raman spectra of the ethyl esters of oleic, elaidic, linoleic, linolelaidic, and linolenic acids. Theoretical considerations lead to the expectation that the *cis* double bond should absorb strongly at 6·0μ, but that the *trans* double bond should not. They reported the ratio of transmission of these compounds to that of ethyl stearate from 5 to 6·8μ. The use of ethyl stearate "blank" was apparently to cancel out the strong ester C=O band in this region. The elaidate and linolelaidate showed very little absorption, while the oleate, linoleate, and linolenate showed strong bands at 6·0μ. Thus, the elaidinized acids were considered to be *trans*, and the natural acids *cis*. Raman spectra data were in agreement with these assignments. Because of the difficulty of working in the 6·0μ region due to ester or acid C=O absorption in this region, this method of studying *cis-trans* isomers has not been further develcped, but a method based on absorption by *trans* double bonds at 10·3μ has been extensively studied.

Isolated (non-conjugated) *trans* double bonds have a sharp and strong absorption band at about 10·3μ, whereas isolated *cis* double bonds do not absorb in this region. Absorption at this wavelength is, therefore, quite valuable in qualitative and quantitative determination of *cis*, *trans* structure in unsaturated acids.

SWERN, KNIGHT, SHREVE, and HEETHER[10] developed a method for determination of *trans* octadecenoic acids and related compounds, based on quantitative measurement of absorption at 10·36μ. Extinction coefficients were reported for the free acids, methyl esters, triglycerides and derived alcohols of a number of saturated and *cis* mono-olefinic acids, as well as the *trans* isomers. The same authors compared the infrared method with the lead salt alcohol method of determining *trans* octadecenoic acids in mixtures with *cis* isomers and saturated acids. The infrared method was more rapid, specific and accurate. It could be used on esters, oils, or the related alcohols without converting to the acid. They noted that an oleic acid, from which the poly-unsaturates had been removed by thermal polymerization, contained 40–45% *trans* acids. An oleic acid prepared from selectively hydrogenated tallow contained 25% *trans* acids, whereas an oleic acid prepared from red oil by distillation and crystallization contained about 4%.

JACKSON and CALLEN[11] also compared the lead salt-alcohol with the infrared method and confirmed the greater accuracy and convenience of the infrared method. They also observed that hydrogenated oils contained from 7·5 to 45% of *trans* or "iso-oleic" acids.

RAO and DAUBERT[12] prepared pure vaccenic acid (*trans* 11-octadecenoic acid) from beef tallow and compared it with oleic and elaidic acids by infrared. The vaccenic acid and the elaidic acid both showed a strong band at 10·25μ which was absent from the oleic acid spectrum. This confirmed the *trans*

configuration of vaccenic acid which is apparently the only known naturally occurring non-conjugated *trans* fatty acid.

AHMAD, BUMPUS, and STRONG[13] synthesized *cis* 11- and *trans* 11-octadecenoic acids. The *cis* acid was synthesized by selective reduction of the corresponding acetylenic acid. The *cis* acid was converted to the higher melting *trans* acid by heating with selenium. The *trans* acid was shown to be *trans*, and identical with RAO and DAUBERT's vaccenic acid from beef tallow, by infrared curves, and by mixed melting points.

LEMON and CROSS[14] partially hydrogenated linseed oil, converted to methyl esters, and isolated an isolinoleate by chromatography. It was shown to contain a *trans* double bond by infrared absorption at 10·3μ.

WOLTEMATE and DAUBERT[15] followed the hydrogenation of methyl β-eleostearate by infrared. The principal changes were the disappearance of the 10·0μ band due to the conjugated triene system, and the appearance of a band at 10·3μ due to *trans* 11-octadecenoic acid (vaccenic acid) which they isolated from the partially reduced ester. They also followed the development of conjugated diene by ultraviolet absorption, and of 1,4-diene by alkali conjugation. Appreciable conjugated diene was formed, but only minor amounts of 1,4-diene. Since the principal mono-unsaturated acid formed was 11-octadecenoic acid, it was concluded that the hydrogenation was fairly selective in that 1,6 addition occurred, followed by 1,4 addition. It was non-selective in that it was not stepwise. They commented on the absence of a 10·3μ band in the original eleostearate which they expected in view of the fact that β-eleostearate was reported to be *trans*. This expectation is in error, since the 10·3μ band is characteristic of isolated, but not of conjugated *trans* double bonds.

FEUGE, PEPPER, O'CONNOR, and FIELD[16] followed the development of *trans* isomers during the hydrogenation of methyl oleate and triolein. They reported molecular extinction coefficients for the methyl and glyceryl esters of oleic, elaidic and stearic acids. As much as 38% *trans* isomer was formed while the first 10% of stearate was formed. Maximum per cent *trans* was 40–48% when 20–30% of methyl stearate was formed. The per cent *trans* was increased by increased temperature, increased catalyst concentration, and decreased degree of dispersion of the hydrogen. Equilibrium between *cis* and *trans* forms always resulted, with 67% of the total unsaturated content in the *trans* form.

REBELLO and DAUBERT[17] studied the isolinoleate formed by partial hydrogenation of normal linolenate. They separated it by low temperature crystallization and showed by infrared that it contained at least one *trans* double bond and that it was a mixture of at least three isomers with the double bonds at the 8,14, the 9,15, and the 10,14 positions.

KHAN, DEATHERAGE, and BROWN[18] used infrared to demonstrate the absence of *trans* double bonds in a synthetic methyl 9,10-dideutero-oleate, and they observed a band at 4·48μ due to the C—D bond. The compound was synthesized by catalytic addition of D_2 to stearolic ester.

MAX and DEATHERAGE[19] used infrared to confirm the CD_2 structure (doublet at 4·64 and 4·84μ) in a synthetic 8,8,11,11-tetradeutero-*cis*-9-octadecene. Their

curves also demonstrated the absence of *trans* double bonds. The compound was synthesized by catalytic hydrogenation of the corresponding tetradeuteroacetylenic compound.

PASCHKE, JACKSON, and WHEELER[20] used infrared in a study of the thermal polymerization of isomers of methyl linoleate. Debromination linoleate was shown to contain a small amount of *trans* isomer. A methyl *cis*-9, *trans*-12 linoleate isomer was isolated by crystallization of the non-conjugated esters of dehydrated castor oil acids. Its absorption at 10·32μ was very nearly the same as methyl elaidate and very nearly half that of methyl linolelaidate. They also showed that *trans* isomers were formed rather rapidly from the *cis*, *cis* linoleate at bodying temperatures, since the recovered monomers showed a fairly strong bond at 10·32μ. The dimers all showed a moderate bond at 10·32, indicating an isolated *trans* double bond.

KNIGHT, EDDY, and SWERN[21] used infrared to demonstrate that *trans* double bonds were formed during the autoxidation of methyl oleate in the presence of ultraviolet light. The increase in absorption at 10·31μ in relation to the peroxide value indicated that the molal concentration of *trans* material was 90% of the molal concentration of peroxide oxygen, up to about 20% oxidation (to monoperoxide). This suggested that most of the peroxides formed are peroxides of *trans* isomers of octadecenoates.

Conjugated cis, trans isomers

JACKSON, PASCHKE, TOLBERG, BOYD, and WHEELER[22] reported infrared studies on conjugated and non-conjugated isomers of methyl linoleate in the region of 10–11μ of olefinic C—H bonding. They showed that the small amount of *trans* isomer present in debromination linoleate could be removed by crystallization. They observed a weak band at 10·95 in non-conjugated *cis*, *cis* linoleate which was not present in the other isomers. They found a single very strong band at 10·12μ characteristic of conjugated *trans*, *trans* linoleate. Conjugated *cis*, *trans* linoleate showed a doublet with a strong band at 10·18μ and a somewhat weaker band at 10·52μ.

PASCHKE, TOLBERG, and WHEELER[23] used infrared in a study of the *cis*, *trans* isomers of the eleostearates. β-eleostearate was shown to be *trans*-9, *trans*-11, *trans*-13 octadecatrienoate, based on physical properties and reactivity as a diene in the Diels-Alder reaction. The β-isomer showed a single very strong band at 10·07μ in the 10–11μ region, similar to the single strong band at 10·12 of conjugated *trans*, *trans* linoleate. The maleic anhydride adduct showed an isolated *trans* double bond (band at 10·32μ) confirming the all-*trans* structure of β-eleostearate.

α-Eleostearate was shown to have the *cis*-9, *trans*-11, *trans*-13 structure, based on the positional structure of the maleic anhydride adduct (addition across the 11,13 double bond pair) and the fact that the maleic anhydride adduct did not show an exocyclic *trans* double bond. The α-isomer had a doublet with a very strong band at 10·09μ and a weaker band at 10·37μ, analogous to the doublet of conjugated *cis*, *trans* linoleate at 10·12 and 10·54μ. Pseudo-eleostearate (10,12,14

isomer) was like the β-isomer in having a single very strong band at 10·07 and was, therefore, considered to have the all-*trans* structure. α- and β-licanic acids (4 keto 9,11,13-octadecatrienoic) were considered to have the same *cis, trans* configuration as the β- and α-eleostearates, by analogy of their maleic anhydride adducts, but were not examined by infrared. Punicic acid is a *cis, trans* isomer of the α- and β-eleostearate (9,11,13-double bonds) occurring in pomegranate seed oil. Its non-identity with α- or β-eleostearate was confirmed by observation of a doublet at 10·08 and 10·61μ in the spectrum of pomegranate seed oil.

Von Mikusch[24] reported the isolation from dehydrated castor fatty acids of an 8,10-conjugated linoleate which was assigned the *trans, trans* configuration on the basis of its reactivity with maleic anhydride and of its melting point. The *trans, trans* structure was confirmed by infrared spectrum on a sample of the same isomeric acid ester furnished by Von Mikusch to Wheeler and Tolberg.[25]

Privett, Lundberg, Khan, Tolberg, and Wheeler[26] used infrared to determine the *cis, trans* configuration of the conjugated hydroperoxide formed from normal methyl linoleate in the early stages (10% peroxide formation) at 0° and at 24°. The peroxide formed at 0° was quantitatively separated from unoxidized linoleate by liquid-liquid extraction (heptane-90% methanol). The theoretical peroxide content and other analyses indicated it to be a rather pure monohydroperoxide. Infrared absorption showed bands at 10·18 and 10·52μ identical in shape and intensity to the same bands found in pure *cis, trans* conjugated (unoxidized) linoleate. These data and the ultraviolet absorption indicated that the hydroperoxide was at least 90% *cis, trans* conjugated structure. Apparently the double bond which moves to form conjugation during hydroperoxide formation goes to the *trans* form almost exclusively. The peroxide isolated from the sample oxidized at 24° showed a considerable amount of *trans, trans* conjugated isomer along with the *cis, trans* conjugated isomer.

Effects of liquid and solid states on infrared spectra of fatty acids and related compounds

Sinclair, McKay and Jones[1] reported on the infrared spectra of saturated fatty acids and esters and some related compounds. In solution, the bands were broad compared to those observed on solid films or mulls. Qualitatively, they were all very similar in solution. The band at 13·9μ due to CH_2 rocking vibration (chain of 4 or more CH_2's) increased progressively with chain length. The crystalline acids (films or mulls) showed much more structure, particularly in the region 7·4 to 8·5μ, where a progression of bands appeared, spaced at fairly even intervals of 0·05μ, apparently due to CH_2 wagging and/or twisting. The position and intensities of these progressions of bands was affected by the polymorphic crystalline form of the acids. The ester C=O stretching vibration occurred at 5·88 to 5·89μ in the even carbon acids, but at 5·87 in the odd carbon acids (crystalline state). In the esters it was at 5·75μ in both solid state and in solution.

Jones, McKay, and Sinclair[27] further investigated the progression of bands

from 7·4 to 8·5μ in the infrared spectra of fatty acids and related compounds in the solid state (mull or film). In the saturated acids from C_{12} to C_{21} there was a regular increase in the number of bands in the progressions. There was no alteration between acids with odd and even numbers of carbons. Hexadecanol and octadecanol showed evidence of two overlapping series of weaker bands. Well defined progressions were observed in 1-iodo-octadecane, dioctadecyl thioether, and dioctadecyl disulphide. In 2-bromostearic acid the bands of the progression were clear and sharp, whereas in 9,10,12,13-tetrabromostearic acid a number of bands of irregular spacing and intensities were observed, not characteristic of a progression. Elaidic acid showed a progression similar to shorter chain saturated acids, whereas solid films of oleic, linoleic and linolenic acids (at liquid nitrogen temperature) showed a number of bands of irregular spacings and intensities.

The bands in these progressions were believed to be due to wagging and/or bending mode of vibration of the C—H bonds of the methylene groups, the number of bands increasing with chain length as a consequence of coupling of the vibrations of adjacent methylene groups. Polar end groups might affect either of these two modes of vibration. The possibility of intermolecular forces or a combination of internal vibrations with lattice vibrations was mentioned as contributing to these bands.

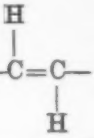
Sinclair, McKay, Myers, and Jones[28] described infrared spectra of a number of unsaturated fatty acids, methyl esters and brominated derivatives as liquids (or solutions) in comparison with their spectra as solids (low temperature films or mulls). Absorption specific to the ethylenic bond was observed in three regions: (*a*) at 3·31μ, attributed to C—H stretching vibrations of C=C—H; (*b*) at 6·06 to 6·34μ, due to C=C stretching vibrations; (*c*) 10·2 to 14·5μ due to out-of-plane vibration of C—H of C=C—H.

The band at 3·31μ was absent from stearic acid, but increased in intensity in the series oleic, linoleic, linolenic, arachidonic. Bands at 3·43 and 3·51μ due to $-CH_2$ vibrations decreased in the same series, and weaker bands at 3·38 and 3·49μ due to the terminal $-CH_3$ group did not change much. The absorption at 3·43 (due to CH_2 groups) decreased while that at 3·31 (related to unsaturation) increased in this series of *cis*, non-conjugated unsaturated acids. A plot of density at 3·43μ divided by the density at 3·43μ minus that at 3·31μ gave a straight line when plotted against number of double bonds. Elaidic acid, however, showed the same ratio as palmitic and stearic acid. This relationship is, therefore, limited to *cis*, unsaturated acids, and presumably to the methylene-interrupted type of unsaturation which occurs in most natural non-conjugated poly-unsaturated acids.

The weak C=C stretching band at 6·03μ was observed on the shoulder of the strong acid C=O band at 5·86μ or ester C=O band at 5·75μ in liquid films. Cooled solid films of the normally liquid unsaturated acids shifted the C=O band to 5·95μ and completely obscured the C=C band. Methyl elaidate (in solution) failed to show the C=C band at 6·03μ, while methyl oleate showed it. Its absence in the *trans* compound was explained on the basis of symmetry of the

trans compound, compared to the *cis* isomer. This effect might not be true if the *trans* double bond were not near the centre of the chain.

In solutions of *cis*- and *trans*-2-octadecenoic acids, the C=O band was shifted to 5·90μ due to conjugation with C=C. The C=C stretching band was greatly enhanced in intensity in both the *cis* and *trans* isomer, due to conjugation with C=O. It occurred at 6·05μ in the *trans* isomer and at 6·08μ in the *cis* isomer, compared to its normal position at 6·03μ.

The band at 10·30μ due to C—H bending of *trans*  was observed on both elaidic acid and methyl elaidate in solution and on elaidic acid solid film. No bands near 14·5μ were observed in the liquid film of oleic acid, as was expected for the C—H of a *cis* . When the film was solidified a strong peak occurred at 14·2μ, tentatively assigned to *cis*-ethylenic hydrogen vibration.

The *trans*-2-octadecenoic acids and methyl esters showed a prominent band at 10·2μ while the *cis* isomer showed a band at 12·1μ. It was suggested that these were due to ethylenic C—H bending vibrations.

A strong band at 6·85μ, present in all of the acids was assigned to unperturbed methylene bending vibration.

A band at 7·10μ was ascribed to the bending vibration of a methylene adjacent to COOH (absent from *cis*- and *trans*-2-octadecenoic acids).

In the unsaturated acids, a band at 6·97μ was observed which increased in intensity with increased unsaturation. It was tentatively assigned to a bending vibration of a methylene adjacent to C=C.

In 2-bromostearic acid the C=O band was shifted to 5·83μ (from 5·80 in stearic acid). In the other bromo acids the bromine is remote and has no effect on C=O frequency. Between 7·4 and 14·3μ the polybromo acids related to linoleic, linolenic, and arachidonic acid show marked differences from one another. Bands between 14 and 20μ were observed and it was suggested that they are due to C—Br stretching vibrations.

Solid films of oleic and linoleic acids showed bands between 13 and 15μ of such a nature that analysis of mixtures might be possible if a series of standard known mixtures were used for comparison.

Monoglycerides

KUHRT, WELCH, BLUM, PERRY, and WEBER[29] used infrared, along with other methods to characterize monoglycerides which were isolated from bread. They also prepared monoglycerides synthetically for comparison. This was essentially a "fingerprint" comparison to show identity, but a rather complete assignment of observed bands was made. They showed that monoglycerides were formed to an appreciable extent when monoglyceride-free fat was used in making the bread.

KUHRT, WELCH, BLUM, PERRY, WEBER, and NASSET[30] similarly used infrared to characterize the monoglycerides formed from triglycerides in the intestinal tract of humans. The recovered lipids showed 40–50% of monoglycerides.

Hydroperoxide groups

SHREVE, HEETHER, KNIGHT, and SWERN[31] studied a number of hydroperoxides and peroxides intended to serve as reference data for analysis of autoxidation products of fats and oils. The hydroperoxides examined (cumene, *t*-butyl, cyclohexene, and tetralin) each showed a band near 2·8μ due to O—H stretching vibration. They also showed a band at 11·82 to 12·01μ which was ascribed to the —OOH group vibrating as a unit, or to vibrations within the group. However, a hydroperoxide of methyl oleate failed to show a distinct band near 12μ as expected. Only a weak, broad band at 11·65μ was observed. It did show the 2·8μ band characteristic of O—H stretching.

Epoxy compounds

SHREVE, HEETHER, KNIGHT, and SWERN[32] reported infrared spectra of some epoxy compounds also intended to serve as reference data for analysis of autoxidation products of fats and oils. Epoxy derivatives of terminally unsaturated compounds showed two bands, near 11 and 12μ. Epoxy derivatives of *cis* monounsaturated compounds showed a band at 11·8 to 12·0μ, while those of the *trans* isomers showed a band at 11·2 to 11·4μ.

Thermal polymerization of 9,10-epoxystearic acid was followed by infrared. The epoxy band at 11·8μ disappeared, as well as the O—H band of —COOH at 10·9μ. A new band at 2·8μ indicated appearance of alcoholic OH groups, and increased absorption near 8μ indicated increased ester content. These changes agreed with the expected reaction of the carboxylic acid group with the epoxy group to form hydroxy, acyloxy structures.

Autoxidation of unsaturated oils

GAMBLE and BARNETT[33] examined some drying oil films before and after extensive oxidation as films under the influence of ultraviolet light. The unoxidized oils showed bands as follows:

3·4μ: C—H (stretching).
5·85μ: C=O (stretching, ester).
6·9μ C—H (bending).
8·4μ: "oxygen in some form" (C—O—C of ester).
10·0μ: conjugation, in tung oil.

The oxidized oils showed the following changes. The 3·4μ band shifted to 3·1μ. This was attributed to an unresolved combination of the original 3·4μ C—H band and a band at 2·9μ due to OH groups. The 5·8μ band of ester C=O increased in intensity. The 10·0μ band in tung oil disappeared, showing loss of conjugation. They commented on the possibilities of using infrared to study pigmented films.

DUGAN, BEADLE, and HENICK[34] studied methyl linoleate which had been oxidized at 98° with air to various peroxide values up to 940 milliequivalents per kilogram (approx. 0·15 mol of hydroperoxide per mole of linoleate). They used a lithium fluoride prism and confined their studies to the regions 2·8 to 3·1μ (OH and OOH region) and 5·68 to 5·90μ (C=O region). With increasing degree of oxidation, bands appeared at 2·88μ and at 2·92μ. The 2·88μ band appeared first, but the 2·92μ band increased more rapidly in later stages of oxidation. The 2·88μ band was assigned to OH groups, since it persisted after reduction of the —OOH group to —OH, while the 2·92μ band was assigned to the —OOH group, since it disappeared on reduction. The reduced peroxides developed a band at 2·82–2·83μ which was ascribed to —OH also. The ester C=O absorbed so strongly at 5·73μ that it largely masked other C=O groups. This effect was overcome by using unoxidized linoleate as a "blank." By this technique, they observed formation of bands at 5·80μ, 5·76μ, and a weak band at 5·70. They suggested that the 5·80μ band was due to C=O groups conjugated to a double bond, and that the 5·76μ band was due to non-conjugated C=O. The weaker 5·70 band was suggested as due to aldehydes from scission products. They also chromatographed the oxidized linoleate and obtained fractions much richer in oxidized materials.

HONN, BEZMAN, and DAUBERT[35] used infrared to determine the increase in —OH groups during autoxidation of linseed oil. The oil was blown with air at 85° for 9·5 hr to a peroxide value of about 400, and sampled at frequent intervals. The samples were analyzed for optical density at 2·9μ as an indication of —OH concentration. The observed values were corrected for contributions of —OOH and —COOH at 2·9μ. Methyl oleate hydroperoxide and palmitic acid were used as standards for calculating the corrections from the determined peroxide and acid values. The corrected density at 2·9μ, attributed to R—OH groups, showed an induction period of 6 hr and then increased rapidly. The peroxide values showed the same induction period but reached a maximum in 8·5 hr and then decreased while the —OH concentration continued to increase. Acid content remained fairly low and contributed little to absorption at 2·9μ. These results indicate that —OH groups are formed simultaneously with —OOH groups under these conditions of oxidation. The possible usefulness of absorption measurements at 2·9μ is suggested as a rapid means of determining the end of the induction period. From their data, an estimation of actual —OH concentration formed during the oxidation would correspond to the formation of an —OH group on about 25% of the fatty acid groups present, in the final 9·5 hr sample.

KRONSTEIN[36] reported some infrared curves on various drying oils, and various gelled oils and separated solid gel portions. The spectra were limited to the 9 to 11μ region. No attempt was made to assign infrared bands to any chemical structures.

ADAMS, AUXIER, and WILSON[37] studied the changes in infrared spectra from 2·5 to 3·7μ of films of synthetic drying oils. Films (0·0015 in. thick) of the dipentaerythritol esters of oleic, linoleic and linolenic acids were spread on a

rock salt slab and were examined after various periods of air-drying. Methyl oleate hydroperoxide and dipentaerythritol stearate were also examined for comparison. The C—H stretching bands at 3·40μ and *ca.* 3·49μ were resolved in all the compounds (except the stearate, which was done in Nujol mull). In the unsaturated esters, before oxidation, a band at 3·27μ was observed which did not show in the stearate or in oleate hydroperoxide. This band increased in intensity from oleate to linoleate to linolenate. It was attributed to a C—H bond frequency peculiar to a carbon adjacent to a double bond (i.e., "active methylenes"). As oxidation proceeded, this 3·27μ band gradually disappeared. Concurrent with its disappearance, a band at 2·78 to 2·82μ appeared with increasing intensity. This was ascribed to —OOH formation. The rate of loss of the 3·27μ band and the rate of increase of the 2·8μ band increased in the order oleate, linoleate, linolenate. The results were considered in accord with FARMER's oxidation mechanism involving formation of hydroperoxide on the carbon adjacent to a double bond. Unfortunately analyses were not made for —OOH by chemical means, so it is difficult to estimate how much of the absorption at *ca.* 2·80μ was due to —OOH and how much to —OH groups.

LEMON, KIRBY, and KNAPP[38] studied the autoxidation of methyl esters of peanut oil fatty acids at 22°, 40°, 60°, 80°, and 100°, and with added iron stearate catalyst at 22°, 60°, and 100°. In the —OH region, a band at 2·92μ was ascribed to —OOH, being approximately proportional to —OOH determined chemically, and appearing first during oxidation. Subsequently, bands appeared at 2·90, 2·86, and 2·84μ ascribed to OH compounds formed by peroxide breakdown. The absorbing compounds could be concentrated (as residue) by high-vacuum distillation, or by counter current extraction. In the C=O region, the ester C=O at 5·75μ was eliminated in the spectra of oxidized samples by using unoxidized ester as reference. The following bands were formed as oxidation progressed: (1) a band at 5·815μ which appeared first and progressively increased (possibly due to C=O conjugated with C=C); (2) a band at 5·85μ which appeared in highly oxidized samples (due to —COOH formed by oxidation); (3) a band at 5·83μ which appeared in highly oxidized samples; (4) a weak band at 5·79μ observed in a very highly oxidized sample. In the region from 9 to 13μ, a band developed at 10·12μ. This band was ascribed to conjugation, since there was a good correlation between it and the ultraviolet diene absorption at 232 mu, as well as with the absorption at 2·92 of the —OOH group. This is in agreement with the formation of conjugated hydroperoxide structure shown by others. A band at 10·27μ appeared slowly. It was ascribed to *trans* double bonds. A weak band at 10·69μ was ascribed to —COOH groups. Highly oxidized samples also showed broad bands at 12·18μ and 11·13μ and a strong band at about 9·36μ, to which no assignments were made.

NICHOLLS and HOFFMAN[39] studied the drying of films (0·03 mm) of pentaerythritol ester of linseed oil fatty acids, glycerol linoleate, pentaerythritol linoleate, blown linseed ("O" viscosity GARDNER-HOLT) and heat polymerized linseed oil ("L" viscosity). The films were dried at 25°, 110°, and 175° for various

times in the dark. Bands were observed and assigned as follows on unoxidized oils:

3·45μ C—H stretching.
5·75μ C=O.
6·85μ —CH_2— bending.
7·10μ —CH_3— bending or —CH_2— bending.
8·0 μ / 8·45μ } C—O of COOR.
9·70μ pentaerythritol skeleton.
10·8μ / 11·5μ } carbon chain skeletal vibrations influenced by double bonds.
6·1 μ C=C.

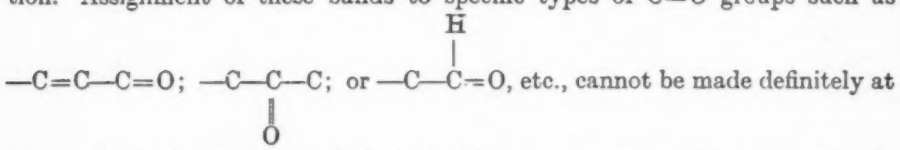
As oxidation progressed, a band at 2·90μ progressively increased. It was ascribed to —OH and —OOH groups. The ester carbonyl band at 5·75μ increased in intensity and widened. The band at 7·9–8·0μ increased and split into two bands after prolonged exposure. This was ascribed, speculatively, to ester C—O or possible to epoxide. A band appearing at 9·20μ was attributed to ester, ether, or secondary OH groups. A band appearing at 10·1 to 10·2μ was ascribed to *trans* double bond or possibly epoxide. The changes occurring on drying at higher temperatures were quite similar but more rapid. In the later samples at 175°, a band at about 6·1μ was observed as a shoulder on the widened ester C=O band at 5·75μ. It was ascribed to C=O conjugated to C=C. The changes observed with driers were generally similar to those without drier. The whole region from 7μ upward became generally opaque in the more highly oxidized films, obscuring any specific structural bands.

They followed the changes in intensity of the —OH band at 2·9μ by taking the ratio of density at 2·9μ to the density at 3·40μ (C—H peak), assuming that the concentration of C—H bonds would not change appreciably during oxidation. This ratio, $\frac{D_{OH}}{D_{CH}}$, increased relatively rapidly to a maximum and then remained fairly constant. The "touch dry" point occurred at about the time of maximum $\frac{D_{OH}}{D_{CH}}$. The change in C=O was similarly followed by taking the ratio of density at 5·75μ to the density at 3·40μ. This value, $\frac{D_{CO}}{D_{CH}}$, also showed an inflection at about the "touch dry" point, but often continued to increase thereafter for some time.

The maximum value of $\frac{D_{OH}}{D_{CH}}$ decreased with increasing temperature, while the maximum $\frac{D_{CO}}{D_{CH}}$ increased. Driers increased the rate of increase of both values. The maximum value of $\frac{D_{OH}}{D_{CH}}$ increased when driers were present. The maximum value of $\frac{D_{CO}}{D_{CH}}$ was decreased by cobalt or lead used singly, but was unchanged when lead-cobalt were used in combination.

These studies on the infrared spectroscopy of autoxidizing drying oils may be summarized as follows: One of the principal effects observed is an increase in absorption at 2·9μ, due to —OOH and —OH groups. Bands due to these two groups are quite close and not ordinarily resolved when both are present. The —OH band is at a slightly shorter wavelength than the —OOH band. The —OOH group absorbs more strongly than the —OH group. Therefore, quantitative measurements at 2·9μ as an overall indication of —OOH plus —OH can be misleading unless this fact is realized.

In the C=O region, the strong band at 5·75μ due to ester —C=O becomes more intense and widened, with a number of other bands appearing. These are not always resolved in the presence of the strong ester —C=O band. The increase in intensity cannot be ascribed entirely to increased ester content with any great certainty, but is quite reasonable. The other bands are probably due to carbonyl groups formed by peroxide decomposition or other types of oxidation. Assignment of these bands to specific types of C=O groups such as



present, but only suggested. Isolation of the specific compounds responsible for these other bands, combined with chemical evidence, and infrared curves for similar known compounds could lead to more definite assignment of these bands to specific structures.

Absorption in the 10 to 11μ region is very probably due in part to formation of isolated *trans* double bonds (10·34μ) and to conjugated dienes (10·12μ and 10·55μ), but much general absorption which is developed in this region cannot be definitely assigned to any specific structure.

The decrease in the band at 3·30μ is quite probably caused by a decrease in content of methylenes adjacent to double bonds by conjugation during peroxide formation, and by subsequent oxidative reactions which destroy the double bonds.

Oil content of oil-styrene copolymers

The strong ester C=O band at 5·75μ (or acid band at 5·85μ) is well suited for detecting and determining the oil content of oil-styrene copolymers, since polystyrene has little absorption in this region.

Brunner and Tucker[40] used the acid C=O bond at 5·9μ to detect fatty acids in fractions of oil-styrene reaction mixtures which had been saponified and fractionated. Tung oil and 11-poise dehydrated castor oil were heated with styrene in xylol at 140°, saponified and fractionated. They concluded that the eleostearate of tung oil did form styrene-oil copolymers, but that the 9,12 linoleate and the 9,11 linoleate did not form copolymers under the conditions used.

Bezman and Browning[41] used the ester C=O band at 5·8μ to determine the oil content of copolymers made in aqueous emulsions of styrene with soy and

linseed oils which had been oxidized by air under various conditions. The unoxidized oils did not copolymerize. The oxidized oils did copolymerize, roughly proportional to the amount of oxidation. Peroxides, conjugation, and increased molecular weight of the oxidized oils were possible contributing factors promoting copolymerization.

HARRISON and TOLBERG[42] used infrared to measure the fatty ester content of polystyrenes made with benzoyl peroxide catalyst at 70° in the presence of the methyl esters of pure stearic; oleic; normal linoleic; normal linolenic; 10,12 linoleic and alkali conjugated linoleic acids. The ester content of the isolated polystyrenes was determined by infrared absorption of fatty ester C=O at 5·75μ, corrected for appreciable absorption due to benzoate from the catalyst and for minor absorption due to the polystyrene. The benzoate C=O showed its peak at 5·70μ. Methyl and ethyl benzoate were used as standards for the benzoate, methyl stearate for the fatty ester, and heat-polymerized, carbonyl-free polystyrene for the polystyrene. Measurements at 5·70 and 5·75μ permitted calculation of fatty ester and benzoate content of the polystyrenes. Molecular weights and polymerization rates were also determined. Methyl stearate and methyl oleate acted largely as diluents, slowing the rate slightly but not reducing the molecular weight of the polymer, and not combining with the polymer to any appreciable extent. Methyl linoleate and linolenate acted as chain terminators, reducing the molecular weight and the rate of formation of the polymer, but entering into the polymer only to the extent of 2 to 3 mol per mole of polymer. The conjugated linoleates copolymerized with the styrene, without affecting the molecular weight or rate of formation of the polymer, but combining to the extent of 12–15 mol per mole of polymer.

Branched long-chain fatty acids

SOBOTKA and STYNLER[43] reported the infrared spectra of normal, iso- (—CH_3 on next to end carbon), anteiso- (—CH_3 on second from end carbon) and neo- (2—CH_3 groups on next to end carbon) palmitic and stearic acids. The iso acids showed a doublet at 7·25 and *ca.* 7·35μ (7·35μ stronger) attributed to the isopropyl group. The anteiso acids showed a band at 7·25 which was stronger than in the normal acids. The neo acids showed the same doublet as the iso acids (7·25 and *ca.* 7·35μ), but the 7·35μ band was stronger than the 7·25μ band. This was stated to be characteristic of the *t*-butyl group present in the neo acids.

FREEMAN[44] reported infrared spectra of 27 branched long-chain fatty acids. Bands at 7·78 and 8·10μ (the former usually stronger) were observed and considered in detail as to the effect of the position of branching on their relative intensities and on minor shifts in wavelength. A methyl in the 2-position made the 8·10μ band stronger than the 7·78μ band. Branching in other positions has less effect on the intensity of the 8·10μ band. Beyond position 8, branching had little effect on these bands. The 8·10μ band was displaced appreciably in the 3-methyl (8·14μ); 4-methyl (8·23μ); 5-methyl (8·25μ); 2,2-dimethyl (8·30μ) and 4,8,12-trimethyl (8·23μ) octadecanoic acids, and in 2-*n*-butyl-2-ethyl nonanoic (8·21μ); 4-*n*-butyl-4-ethylnonanoic (8·20μ); and 2,3,4-trimethyl

hexadecanoic acids. The intensity of the 13·85μ band (chain of 4 or more unbranched carbons) varied in intensity approximately as structures would predict. Ethyl group substituents showed a band at 12·9μ, *n*-propyl groups showed a band at 13·5μ, isopropyl groups show a band at 8·55μ. Two methyl groups on the same carbon resulted in a splitting of the 7·25μ band. The number of (methyl) branches up to 4 was correlated with optical density at 7·25μ. The range of optical density at 7·25μ for a particular number of methyl groups was distinct except for a slight overlapping between 2 and 3. Branching near the carboxyl group tended to give lower values.

A band at 8·8μ was suggested as being associated with a quaternary carbon beyond the 2 position.

The provisional nature of many of the assignments was recognized by the authors. However, they represent correlations which are of value in structural work if used judiciously, and if correlated with other means of structure identification.

Phthienoic acids from tubercle bacillus

CASON, FREEMAN, and SUMRELL[45] used infrared in studying the structure of a C_{27} phthienoic acid isolated from tubercle bacillus. They proposed its structure to be:

$$\begin{array}{l} C_4H_9\text{—}(CH_2)_{15}\text{—}CH\text{—}CH_2\text{—}CH{=}C\text{—}COOH \\ \qquad\qquad\quad | \qquad\quad | \qquad\qquad\quad | \\ \qquad\qquad CH_3 \quad CH_3 \qquad\quad CH_3 \end{array}$$

(A C_{29} acid was also isolated which was of similar structure, from infrared evidence.) Unsaturation was indicated by a band at 6·08μ. Shift of the ester C=O from 5·75 to 5·85μ was in agreement with the α-β position of the double bond, also indicated by ultraviolet absorption. Bands at 12·5, 13·26, and 14·92μ indicated at least one H on the unsaturated carbons. No splitting of the 7·3 band into a doublet indicated the absence of iso or neo structures. The absence of a band at 8·8 indicated the absence of quaternary carbons. No bands between 12·5 and 13·8μ indicated the absence of ethyl or propyl branches. Absorption at 7·3 was consistent with 4 methyl groups (also determined chemically). The locations of the methyl groups was based largely on chemical-physical properties not related to infrared.

Cyclopropane ring in lactobacillic acid

HOFMANN, LUCAS, and SAX[46] isolated a lactobacillic acid, $C_{19}H_{36}O_2$, m.p. 28–29°C, from the fatty acids of *Lactobacillus arabinosus*. Since the acid was saturated (no reaction with $KMnO_4$ or peracids), and contained 2 less hydrogens than a C_{19} saturated acid, a cyclic structure was suggested. Hydrogenation produced *n*-nonadecanoic acid and what was apparently a methyl octadecanoic acid. Infrared absorption showed a band at 9·8μ which is characteristic of alkyl substituted cyclopropanes (9·9μ) (DERFER, PICKETT, and BOORD).[47] This established the structure as $CH_3\text{—}(CH_2)x\text{—}CH\overset{CH_2}{\text{——}}CH\text{—}(CH_2)y COOH$, where $x + y = 14$.

Cyclopropene ring in sterculic acid

NUNN[48] isolated sterculic acid $C_{19}H_{34}O_2$, m.p. 18–20°, as the main constituent of the kernel oil of *Sterculia foetida*. Ozonization produced 9,11 diketo nonadecanoic acid. Complete hydrogenation consumed 2 mol of hydrogen and produced a saturated acid $C_{19}H_{38}O_2$, m.p. 65·2–66·8°. Partial hydrogenation with 1 mol of hydrogen produced dihydrosterculic acid, m.p. 38·8–39·8° which was a saturated acid (no reaction with $KMnO_4$) showing an infrared absorption band at 9·85μ, characteristic of cyclopropanes. This established the structure as

$$CH_3—(CH_2)_7—CH\overset{CH_2}{\diagup\diagdown}CH—(CH_2)_7COOH.$$

Herculin and related unsaturated n-isobutyl amides

Herculin is one of a number of naturally occurring N-isobutyl amides of unsaturated fatty acids with two or more double bonds. CROMBIE[49] used infrared to reinvestigate the proposed structure of herculin, N-isobutyl amide of 2,8-dodecadienoic acid. He examined the N-isobutyl amides of all four *cis*, *trans* isomers of 2,8-dodecadienoic acid. None were identical with herculin in chemical or physiological properties. He therefore concluded that herculin had been assigned a wrong gross structure.

The structures of the synthesized amides of the four isomeric 2,8-dodecadienoic acids was substantiated by infrared data. A N—H stretching band was observed at 3·04μ in all amides. The C=O band was at 6·14μ, displaced by conjugation with the C=C double bond from its normal position of 6·10μ for the C=O of monosubstituted amides. A band at 6·47μ, characteristic of monosubstituted amides was observed in all isomers. The isomers with the *trans*-2 double bond in conjugation with the amide C=O showed bands at 10·22μ, displaced from its normal position at 10·35μ which was observed with the isomers containing the isolated *trans*-8 double bond. A band at 2·25μ, which was very weak in the *trans, trans* or saturated amide, increased progressively in the *cis, trans* and the *cis, cis* isomers, to a band of moderate intensity.

Stretching frequencies due to the C=C conjugated to C=O were observed at 6·05μ with the *cis*-2 isomers and at 6·00 with the *trans*-2 isomers. The nonconjugated 8-double bond was shown not to contribute to the C=C stretching bands by examination of model compounds containing no conjugated C=C bonds.

Mycomycin

CELMER and SOLOMONS[50] elucidated the structure of the antibiotic, mycomycin as (—) 3,5,7,8-*n*-tridecatetraene-10,12, diynoic acid (probably 3-*trans*, 5-*cis*, or possibly 3-*cis*, 5-*trans*):

$$CH{\equiv}C—C{\equiv}C—CH{=}C{=}CH—CH{=}CH—CH{=}CH—CH_2COOH$$

This most remarkable compound is quite unstable, losing half of its biological activity in 3 hr at room temperature, and exploding at its melting point of 75°. Isolation and manipulations were carried out under inert gas at low temperatures.

They also showed that mycomycin is rapidly converted by dilute aqueous alkali to isomycomycin, *trans*-3, *trans*-5 *n*-tridecadiene-7,9,11 triynoic acid:

$$CH_3—C\equiv C—C\equiv C—C\equiv C—CH=CH—CH=CH—CH_2COOH$$

The proof of these structures is a most elegant example of the use of infrared and ultraviolet spectroscopy, combined with chemical evidence to deduce structure.

Hydrogenation of mycomycin or isomycomycin required 8 mol of hydrogen and produced *n*-tridecanoic acid. The terminal acetylene group in mycomycin was established by its reaction with alcoholic silver nitrate and by a strong infrared band near 3·08μ and a weak band near 4·90μ. The second acetylenic bond in mycomycin was indicated by an infrared band at 4·55μ characteristic of disubstituted acetylenes. The conjugation of the two acetylene bonds was indicated by characteristic ultraviolet spectra. This fixed the two acetylene bonds at positions 10 and 12.

Ultraviolet maximum at 281 mμ limited the number of contributing conjugated multiple bonds to three, parts of which might be acetylenic. Absence of a double bond conjugated to carboxyl was shown by the normal C=O bond at 5·76μ with the methyl ester of mycomycin. The 3,5-diene grouping in mycomycin was suggested from its proven existence in isomycomycin (*cf.* below). Infrared bands at 10·53 and 10·15μ characterized the conjugated diene system as a *cis*, *trans* diene, which explained its inactivity in the Diels-Alder reaction with maleic anhydride. This was in contrast to isomycomycin which had a *trans*, *trans* conjugated diene system (strong band at 10·10μ, but no band at 10·53μ) and which reacted readily with maleic anhydride. These facts established the diene in mycomycin system as 3-*trans*, 5-*cis* or 3-*cis*, 5-*trans*.

The allenic grouping (at positions 7 and 8) was indicated by an infrared band at 5·18. Its position was fixed by the position of the diene at 3 and 5 and of the diyne at 10 and 12. This location of the allene group was substantiated by its explanation of the optical activity of mycomycin, and also of the ultraviolet spectrum with two maxima at 267 and 281 mμ.

The determination of the structure of isomycomycin was closely related to that of mycomycin. It also absorbed 8 mol of hydrogen to give *n*-tridecanoic acid. Infrared showed no allenic and no terminal acetylenic absorption (also shown to be absent by non-reactivity with alcoholic silver nitrate). It analyzed for one C-methyl group, while mycomycin showed none. Its ultraviolet spectrum was similar to known conjugated dienetriynes and lack of conjugation of unsaturation with the ester carbonyl was indicated by the normal position of the ester carbonyl band (5·77μ). Isomycomycin readily added 1 mol of maleic anhydride. The adduct had ultraviolet absorption characteristic of conjugated triynes. The adduct added 7 mol of hydrogen to produce a product which was identical with the hydrogenated adduct of maleic anhydride with 3-*trans*, 5-*trans* *n*-tridecadienoate (which was synthesized). This established the diene in the 3,5 positions, and showed it to be a *trans*, *trans* diene. The greater intensity of the band at 4·55μ (disubstituted acetylene) of isomycomycin compared

to mycomycin suggested a triyne structure, and with the presence of a terminal $—CH_3$ group, limited the triyne structure to position 7, 9, 11. This established the structure shown for isomycomycin.

These authors also synthesized methyl 3-*trans*, 5-*cis*-*n*-tridecadienoate and methyl 3-*trans*, 5-*cis*, *n*-nonadienate as models for their infrared diagnosis of the conjugated diene in mycomycin. These *cis*, *trans* conjugated dienes showed infrared bands at 10·53 and 10·15μ similar to those shown by mycomycin and also similar to those shown by *cis*, *trans* conjugated linoleates. Catalytic isomerization with iodine changed them to the *trans*, *trans* forms which showed a single stronger band at 10·10μ, like that of isomycomycin and like that of *trans*, *trans* conjugated linoleates.

Aluminum soaps

HARPLE, WIBERLEY, and BAUER[51] used infrared to study the structure of the aluminium salts of fatty acids from C_6 to C_{18}. Fatty acid bands (principally 5·82μ of acid C=O) appeared only in those soaps which contained fatty acids extractable with cold iso-octane. The results indicated that trisoaps of the higher fatty acids do not exist as chemical compounds, but as a mixture or loose compound of a disoap and fatty acid. Disoaps and monosoaps show no infrared bands characteristic of the COOH group and almost no extractable acid. Monosoaps show a broad band at 3·0μ, indicating hydrogen bonded OH groups, while disoaps show a band at 2·70μ indicating unbonded OH groups. Soaps of a composition between mono and disoaps showed both the 3·0μ and the 2·70μ bands, indicating that the monosoaps and the disoaps exist as discrete compounds.

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7

COUNTERCURRENT FRACTIONATION OF LIPIDS

Herbert J. Dutton

THE separation of solutes on the basis of their differential solubilities in two immiscible solvents has been known in principle as long as the science of chemistry and has been practised since prehistoric time for the preparation and purification of perfumes and oils. Until comparatively recent times, liquid-liquid extractions have been applied only to materials of widely differing solubility characteristics of such a nature that a single-batch extraction or, at most, multiple extraction of one phase was required to effect practical separations. It is only within the last few decades that the principle of countercurrent flow and the elaboration of contacting stages have been applied to the fractionation of more difficultly separable solutes.

"Countercurrent distribution" is the name given to a particular type of multiple-batch extraction which is carried out in ingenious laboratory devices designed by CRAIG.[1] This technique can be recommended to the lipid chemist for a number of reasons, not the least of which is the mildness of the fractionating conditions compared to distillation, or even to chromatographic procedures. Particularly for the unstable compounds such as fat oxidation intermediates, the low temperature and dilute solution conditions of the procedure gain in importance. Because approximately complete recovery of the starting material in the fractions is obtained, a countercurrent distribution curve has particular value in that it accounts for all the material. The reproducibility and predictability of solute behaviour with this technique, the capacity for expansion both in volume and in resolution, the analytical and preparative potentialities, and the inherent adaptability of partition processes to problems of lipid chemistry should become apparent to the reader.

Although much of the attention of this review is directed toward a description of laboratory countercurrent distribution apparatus, its operation and applications, certain recent commercial developments in the countercurrent extraction of lipids have been made and are briefly considered herein.

FUNDAMENTALS OF COUNTERCURRENT FRACTIONATION

The partition coefficient may be considered as characteristic a constant of chemical compounds as refractive index, melting point, boiling point, and specific rotation. In addition, the partition coefficient has the advantage over many of the familiar physical constants that its consideration may suggest methods of fractionation and isolation.

The partition coefficient (K) is defined by the equation

$$c_1/c_2 = K$$

where c_1 and c_2 are the concentrations of the given solute in the lighter (upper) hyperphase and the heavier (lower) hypophase solvent layers, respectively. The equation follows from HENRY's law and states that the ratio of concentrations of a given solute in two immiscible phases at equilibrium is constant at a given temperature. Deviations occur at high concentration and under conditions where the ratio of associated and dissociated molecules vary. For purposes of the present discussion, the partition coefficient will be assumed to be constant, this constancy of coefficient being consciously striven for in the countercurrent distribution work to be described by using dilute solutions.

The separation of relatively polar and relatively nonpolar solutes on the basis of their differential solubility in immiscible solvents is a common laboratory operation most frequently carried out as a single separatory-funnel operation. The partition of saponified plant pigment extracts between petroleum ether and aqueous alcohol layers into the hyperphasic yellow, unsaponifiable carotenoids and the hypophasic green, saponifiable sodium chlorophyllins constitutes an example of a simple fractionation of lipids achieved by virtue of differential solubilities in two immiscible solvents. However, by definition, lipids are relatively nonpolar substances, and it is not surprising that poor separations are generally achieved by the use of a single equilibration because of the closeness of partition coefficients. Rather a slight fractionation results at a single equilibration, and the application of successive stages is required to effect separation.

Of various possible arrangements for applying multiple stages,[2] countercurrent operation affords the most efficient procedure in terms of volume of solvents used and degree of separations attained.[3] In this scheme of operation, successive portions of one solvent phase moving in one direction come into contact with successive portions of a second solvent phase moving in the opposite direction. Solutes dissolved in the two phases tend to go with one phase or the other, dependent upon their partition coefficients and the volumes of the solvents in contact.

The equilibration of the countercurrently flowing solvents may be achieved in discrete steps, in which case the system is described as multiple stage, countercurrent batch extraction. If the flow of the solvents is continuous, the system may be described as multiple stage, continuous countercurrent extraction.

Multiple stage, countercurrent batch extraction

Since many of the applications of countercurrent fractionation to be described were carried out by countercurrent distribution techniques, an analysis of this procedure will be made. The process is best illustrated by an example involving separation of two solutes. In this instance, successive portions of the upper layer are caused to move over and be equilibrated with equal successive volumes of lower layers. To clarify the relationship of theory to the apparatus actually used for countercurrent distribution, the lower layers will be held stationary. Assume that three separatory funnels, numbered 0, 1, and 2, each contain a given volume of hypophase. To funnel 0 is added an equal volume of hyperphase and 1000 units each of solutes A and B. Assume further for illustrative

purposes that solute A is 9 times more soluble in the hyperphase than in the hypophase, while solute B is 9 times more soluble in the hypophase than in the hyperphase. The partition coefficient for compound A is therefore 9 and that for B is 0·11.

Considering for the moment only compound A, the distribution of 1000 units of A in funnel 0 after equilibration and settling is calculated. As shown in Fig. 1, 900 units will be found in the hyperphase and 100 in the hypophase after the first equilibration. The hyperphase of funnel 0 is next transferred to funnel 1, and a volume of fresh hyperphase solvent is replaced in funnel 0. After shaking and settling, the distribution of solute A is shown at transfer stage 1. In separatory funnel 0, the 100 parts of A left dissolved in the lower layer are now distributed to give 90 parts in the hyperphase and 10 parts in the hypophase. The 900 units transferred to funnel 1 in the hyperphase are now distributed, 810 parts in the hyperphase and 90 in the hypophase.

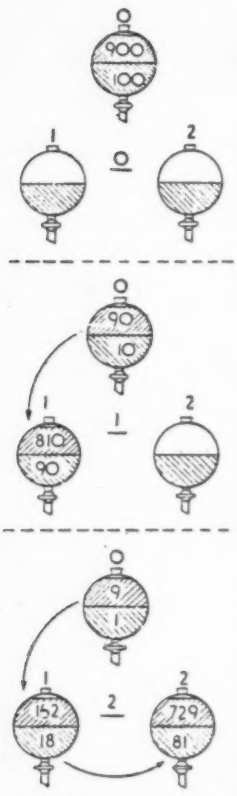

Fig. 1. Countercurrent distribution of solute A ($K_A = 9$).

In the next operation, the hyperphases of funnels 0 and 1 are transferred to funnels 1 and 2, respectively, and fresh hyperphase is again introduced into funnel 0. The distribution after shaking and settling is shown at transfer stage 2 of the figure. The solute A content of each funnel, or the sum of that in the two layers, is 10, 180, and 810 units respectively for funnels 0, 1, and 2.

Solute B, by analogous reasoning, will be found to have the reverse distribution. The solute B content for funnels 0, 1, and 2 is 810, 180, and 10 units, respectively. When both solutes are now considered, funnel 0 is found to contain 81% of solute B of 98·8% purity and funnel 3 contains 81% of solute A of 98·8% purity. This two-transfer-stage operation, just described, demonstrates the countercurrent distribution process and also shows the increased separation of solutes A and B over that for the single equilibration where the corresponding purities of solutes A and B are 90%.

The distribution of solutes in the various stages of a countercurrent distribution can be calculated readily through use of the binomial expansion which describes this process[4]:

$$\left[\frac{1}{K+1} + \frac{K}{K+1}\right]^n$$

where n is the number of transfers.

In the example described above where $n = 2$ and $K = 9$, the expansion is:

$$\frac{1}{(K+1)^2} + \frac{2K}{(K+1)^2} + \frac{K^2}{(K+1)^2} \text{ or } 0{\cdot}01 + 0{\cdot}18 + 0{\cdot}81$$

When multiplied by 1000, the distribution given above is obtained.

A general form of the equation which describes the solute content ($T_{n,r}$) of any given funnel or tube (r) after a given number of transfers (n) is:

$$T_{n,r} = \frac{n!}{r!(n-r)!} \cdot \left(\frac{1}{K+1}\right)^n \cdot K_r$$

Certain shortcuts make the calculation of solute contents in individual tubes, and thus of theoretical distribution curves, quite simple in practice.[4]

The ability to calculate the solute content of each funnel or tube of a countercurrent distribution, when the partition coefficient and the number of transfers

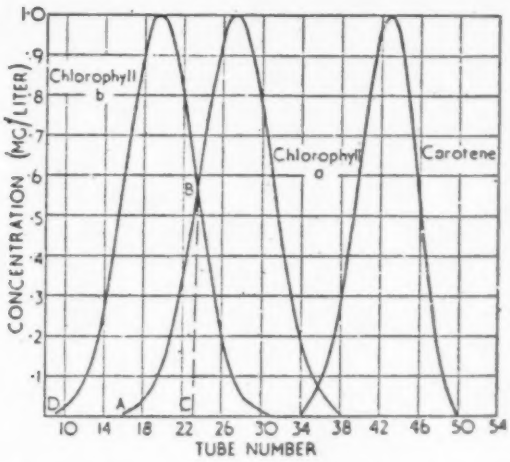

Fig. 2. Theoretical curves of chlorophyll *a*, chlorophyll *b*, and carotene for a 54-tube Craig countercurrent distribution.

is known, constitutes one of the significant advantages of this method of fractionation. This feature suggests the analytical function of the technique. Under conditions of constant partition coefficient, any deviations from the theoretically calculated distribution can be attributed to another solute or solutes. The sum amount of the deviation, of course, represents the amount of the other solute or solutes.

The prediction of the degree of separation of two solutes, A and B, given their partition coefficients (K_A, K_B) is a problem of frequent occurrence. This problem may take the form of a question: either, how many stages need to be applied to achieve a desired purity; or, what separation will be achieved by the application of a given number of stages?

An approximate answer to these questions can be given by statistical theory in which probabilities are calculated from the area of overlap of curves such as are illustrated for the chlorophyll *a* and *b* in Fig. 2. The impurity of chlorophyll *a* in chlorophyll *b* is defined by statistical considerations as the ratio of area ABC to the area BCD. The following relationships hold between various

percentage overlaps or percentage impurities for selected values of the statistical constant t:

t	*Percentage Impurity*
1	15·9
2	2·27
3	0·13

Further data on the relationship of t to the area of overlap are available in the tables of standard statistical texts.[5]

The relationship between partition coefficients, number of transfers, and t is given by the equation:[6]

$$n = t^2 \left[\frac{K_A + K_B + 2K_AK_B}{K_B - K_A}\right]^{2*}$$

A nomographic solution of this equation is presented in Fig. 3. It is useful within the range of partition coefficients normally used and for the number of stages available in the metal-type apparatus described below. Equal volumes of solvent layers are assumed. The solution illustrated in the nomograph is the determination of the number of stages required to achieve 95% purity, given partition coefficients 0·5 and 2·0. Where a larger number of transfers is involved and coefficients are not in the range 0·25 to 4·0, the equation given above can be employed. The function:

$$\left[\frac{K_A + K_B + 2K_AK_B}{K_B - K_A}\right]^2 = R^2$$

may be looked upon as an index of separability for solutes with partition coefficients K_A and K_B; the larger the value of this function (R^2), the greater is the difficulty of separation. R^2 is also the calculated number of transfers which are required to achieve a 84·1% ($t = 1$) separation of solutes A and B. It serves, as shown in following sections, as a useful index for comparing the difficulty in separating various binary systems.

The choice of immiscible solvents for countercurrent distribution, unfortunately, is a largely empirical process. However, certain desirable characteristics of a solvent system may be listed:

1. Particularly for the metal apparatus or for the "fundamental" technique of operation, the partition coefficient for one of the components to be separated should be near 1. For a binary system, greatest efficiency of separation is attained when partition coefficients are reciprocals or their geometric mean is 1.[3]

* The equation derived by NICHOLS[7] is of the same form and apparently gives comparable approximations:

$$n = t^2 \left[\frac{(K_AK_B - 1)}{K_A + 1\sqrt{K_B} - (K_A + 1)\sqrt{K_B}}\right]^2$$

It is to be noted that CRAIG[3] has applied theory of distillation to the prediction of separation, but this requires considerably more labour than that indicated by the above equations.

2. The solvent system should be temperature insensitive, i.e., little change in mutual solubility of the layers and, therefore, little change in the position of the interface should occur with temperature change.

3. Boiling points of solvents should be low enough to permit their ready removal for gravimetric analysis and yet high enough to minimize evaporation, pressure development, and leakage in the instrument.

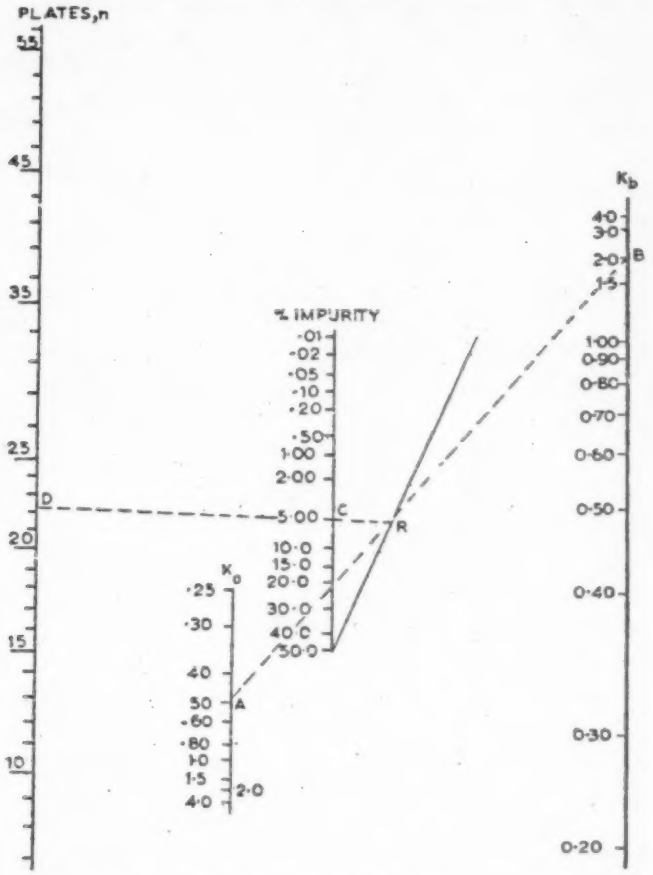

Fig. 3. Nomograph relating plates, n, per cent impurity, K_A and K_B.

4. Differences in specific gravity and lack of emulsifying tendency should be such as to permit rapid separation of the phases.

5. Association between solute molecules should be minimized to give as nearly normal frequency distribution curves as possible.

6. Solvent systems should be selective. This last-named point of selectivity is perhaps the most critical and yet the most elusive. Experience gained through the empirical approach appears to be the best guide to choice of solvents. Suffice it to say that wide differences in separability (R^2 values) in various solvent systems do exist and their advantages should be exploited.

Multiple stage, continuous countercurrent extraction

Commercial applications of liquid-liquid extraction have most frequently followed this type of operation for reasons of the simplicity, the general amenability of continuous processes to commercial exploitation, and the efficiency in terms of solvents used and separations attained. Whether mixing and equilibration stages are established by means of stirrers or packings, settling zones or centrifuges, the essential features are the continuous countercurrent flow of immiscible solvents with multiple contacts. Solutes to be separated are most frequently fed in at the centre of the train and preferentially leave the column with the lighter (raffinate) or the heavier (extract) phase, depending on their individual partition coefficients and the ratio of the volumes of solvents.

The mathematical analysis of this operation is complicated, as has been pointed out earlier, since the overall fractionation obtained is a function of the differential rate of transfer of solutes across solvent boundaries as well as a function of the partition coefficients.[3] High concentrations are most frequently employed, and under these conditions partition coefficients vary. General practice in the analyses of this type of operation is an empirical approach, although some progress in theoretical analysis of this type of operation has been made.[2], [8]

Equipment

Apparatus for countercurrent distribution

Many recent advances in knowledge of lipid chemistry have been made possible by the development of apparatus for efficiently and systematically carrying out the countercurrent distribution operation. In the metal instrument described by Craig and Post,[9] a 24-transfer distribution, for example, is accomplished in less than an hour, an operation which if carried out in separatory funnels would require the performance of 1800 ($3 \times 25 \times 24$) individual shaking, settling, and transfer operations. Such an amount of labour would have discouraged the application of countercurrent distribution processes even if the potentialities of the method had been fully realized.

A drawing of an early model of countercurrent distribution apparatus having 19 tubes is presented in Fig. 4. It consists of two cylindrical stainless steel blocks into which a concentric row of holes has been bored through the uppermost piece and into the lower section. The critical feature of the apparatus is the carefully lapped surfaces between the two sections and between the upper section and cover which permit the upper section to be rotated with respect to the lower section and the whole apparatus to be inverted for mixing of phases without loss of solvent. In operation, the holes of the lower section are filled with hypophase, and an equal volume of hyperphase is introduced into each hole of the upper section. The apparatus consists, in effect then, of a series of 19 separatory funnels arranged in a circle and permits the upper layers of each tube to be transferred to the next adjacent tubes in a single operation. Mixing of the phases of the 19 tubes is similarly accomplished in a single operation by inverting the apparatus about its horizontal axis.

Equipment

Countercurrent distribution is performed by introducing the solutes to be separated into tube O/O of the apparatus which has been filled with the previously equilibrated solvent layers. Mixing, settling, and transfer operations are successively repeated until one revolution of the upper section has been made and upper tube O comes to rest over lower layer 19. Variations of this, so designated, "fundamental"[1] operation procedure will be discussed as they arise. The whole contents of each tube (upper and lower layers) are removed by

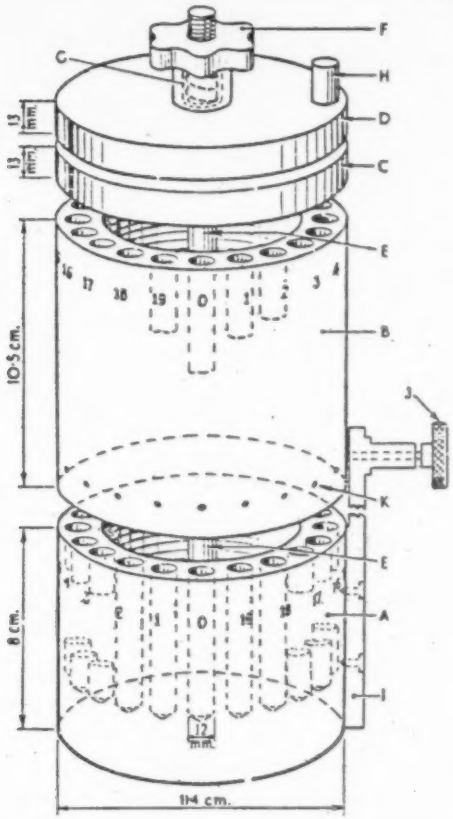

Fig. 4. Metal countercurrent distribution apparatus.

siphoning or by suction; and weight determinations, spectrophotometric analyses, fluorescence intensities, etc., are determined, either on the residues after evaporation of the solvents, or upon aliquots of the tube contents.

One of the newer models of the metal type apparatus has 54 tubes with a capacity of approximately 16 ml per tube and is characterized as an analytical model. Another model designed for preparative purposes has 29 tubes of 1 in. diameter and 160 ml capacity per tube.[9] A feature common to these models is the use of glass plates for bottom and top covers. This is particularly advantageous when working with coloured substances or with substances which tend to give emulsions.

Countercurrent distribution equipment of all-glass construction has been designed and has now attained wide usage. This type of apparatus was made possible by an ingenious design which permits the upper layer of each glass tube to be transferred to the next adjacent tubes merely by tipping slightly more than 90° and then causing the tube to resume its original position. Mixing is achieved by a rocking motion of the tube in horizontal position. Glass tubes are constructed with a volume as large as 200 ml and as small as 20 ml. The tubes are arranged in a linear fashion as shown in Fig. 5. A principal advantage of the glass design is the lower cost of construction per tube, a reduction which means that more stages may be obtained for the same equipment cost. Another advantage includes the possibility of expanding the number of stages by merely adding more tubes to the train.

This glass apparatus may be operated according to the "fundamental" plan in a manner comparable to that described for the metal models, the chief difference in operation being the necessity of adding fresh hyperphase to tube O after each transfer. Where increased numbers of transfers are desirable, the effluent hyperphases may be collected as they flow from the last tube, thus applying increased stages to the low partition coefficient solutes remaining in the apparatus. The operation may be continued until the last-mentioned solutes are finally eluted from the apparatus. Use of the apparatus according to this "single withdrawal principle" is analogous to elution analysis of chromatography. The mathematical description of this process has recently been published.[10] In certain cases, the effluents from the last tube are reintroduced into tube O, and increased numbers of effective tubes are achieved by this re-cycling process.

Increasing the number of extracting stages in order to improve the resolution of difficultly separable solutes results in increased labour even with the labour-saving features of countercurrent distribution apparatus. An answer to this problem is to be found in the automatic equipment designed by CRAIG, HAUSMANN, AHRENS, and HARFENIST.[11] This instrument with its 220 tubes is pictured in Fig. 6. Shaking, transfer, collection of samples, and introduction of fresh solvent are all accomplished automatically. Provision is made for re-cycling, and with this procedure several thousands of stages have been applied to difficultly separable binary systems, the equipment operating continuously over a period of days. A further advantage of this apparatus is that it extends the range of partition coefficients which may be conveniently studied, from the 0·25 to 4 range for metal apparatus, to the 0·01 to 100 range.

Equipment for multiple stage, continuous countercurrent extraction

Whereas the apparatus of the preceding paragraphs is limited in applicability to laboratory scale operations, the equipment described in this section is either of commercial interest or is capable of expansion to commercial scale. Less consideration must necessarily be given this type of equipment, which is primarily of engineering interest. Moreover, the published applications to lipids at the present time are relatively few. Operations of this type will undoubtedly attain

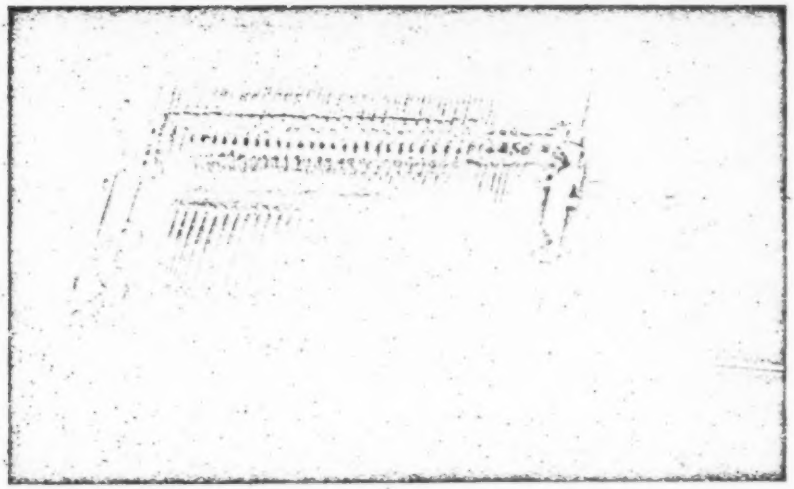

Fig. 5. Glass countercurrent distribution apparatus.

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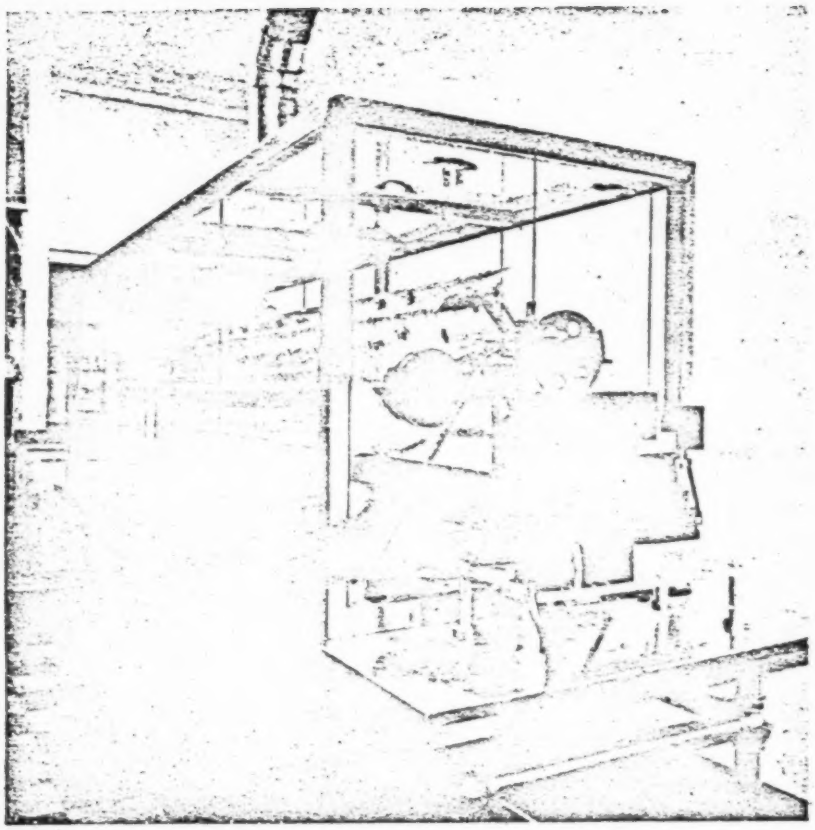


Fig. 6. Completely automatic glass countercurrent distribution equipment.

(To face page 301)

greater interest among industrial chemists as potentialities for liquid-liquid extraction and are suggested by laboratory, multiple-batch experiments. In a rough manner, multiple-stage, continuous countercurrent extraction apparatus may be classified as (1) that in which mixing, settling, and transfer is attained in discrete stages, and (2) that in which mixing, settling, and transfer is a continuous process.

Schematically, the discontinuous mixing-settling systems may be represented by Fig. 7. Hypophase is introduced at one end of a train consisting of alternate

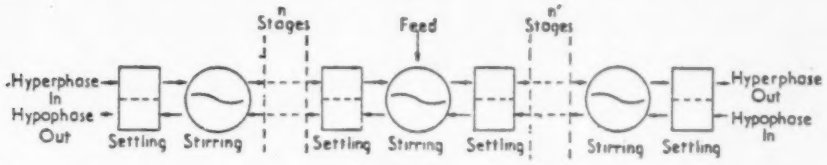


Fig. 7. Schematic representation of multiple stage, continuous countercurrent extraction.

settling and mixing equipment, and the hyperphase at the opposite end. Each phase is, of course, removed at the end opposite from its introduction. The point of entry of solutes to be separated is at some point between the two ends of the train, depending on the particular system. Various types of mixers and separating equipment are used. Gravity separation may suffice in some cases;[12] centrifugal separators are used in others as illustrated in the flow diagram of Fig. 8.[13] Attention is particularly called to the novel centrifugal contactors of

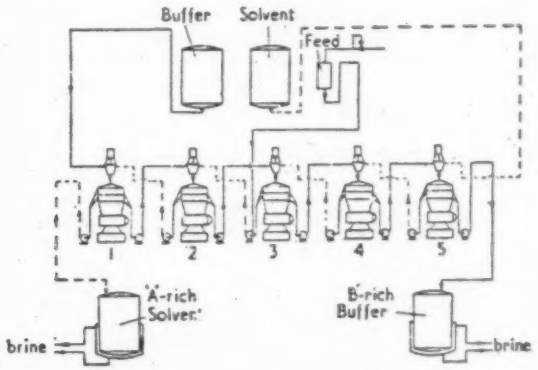


Fig. 8. Countercurrent fractionation with five disc type centrifuges.[13]

the Podbielniak design shown in Fig. 9.[14] Hyperphase is introduced at the periphery of the rotor and hypophase at the centre. Exit of the hyperphase is at the centre and exit of the hypophase is from the periphery of the rotor. As the phases move countercurrently under 2000 to 5000 G., they are mixed in passing through the holes of the perforated concentric rings. Separation of the phases occurs between the rings. Eight or more theoretical stages have been observed for this design. Among the laboratory models of multiple-stage continuous countercurrent extraction apparatus are those of CORNISH, ARCHIBALD,

MURPHY, EVANS,[15] VAN DIJCK, and RUYS,[16] MARTIN and SYNGE,[17] and KNOX, WEEKS, HIBSHMAN, and MCATEER.[18]

Equipment in which mixing, settling, and transfer is a continuous process is generally a vertical columnar type where gravity is the transferring agent. Introduction of the hypophase (extract) is at the top of the column and take-off at the bottom; introduction of the hyperphase is at the bottom with overflow at the top. Feed of materials to be separated may be at any point intermediate between top and bottom. Mixing of the phases with themselves and with the feed is most frequently achieved by packings; e.g. saddles, helices, etc., in the column, though mixing is also accomplished by internal stirrers.

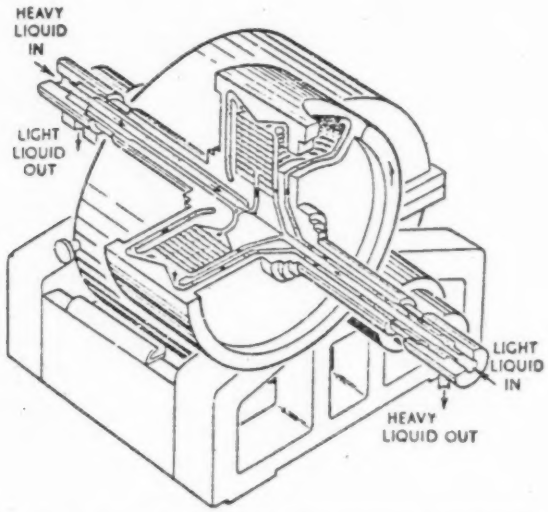


Fig. 9. Podbielniak centrifugal countercurrent contactor.[11]

Two laboratory models of internally stirred equipment merit individual discussion. The column designed by SCHEIBEL is actually of the discontinuous stage type, although it is a vertical column in construction.[19] The laboratory model consists of a glass tube equipped with inlet and outlet tubes for the countercurrent flow of solvents and the introduction of feed. Through the centre passes a shaft with spaced paddle wheels for stirring. Between the paddle wheels are packings of stainless steel mesh which serve to stop the motion of the solvents and permit separation of phases. The lighter phase then moves upward as it separates into the next stirring section, while the heavier phase moves downward into another stirring section. Efficiencies have been reported for this column slightly higher than one plate per stirring and settling section.

The second type of continuous liquid-liquid extraction column is that devised by JANTZEN[20] and most recently evaluated by SHORT and TWIGG.[21] It has a glass column similar to that described for the Scheibel column but has at its centre a spinning cylinder. Under correct operating conditions a banded arrangement of horizontal vortex rings develop. Finely dispersed solvent phases

thus come into frequent contact as they move spirally upward and downward. In a 2-ft column described by SHORT and TWIGG and used for the extraction of acetic acid from water phase with methyl isobutyl ketone, thirty-four theoretical stages were calculated to have been present. An efficiency of nearly this same magnitude was reported by NEY and LOCHTE in earlier work.[22] Since the most efficiently packed columns have a height equivalent to a theoretical plate (H.E.T.P.) of approximately 1 ft, the H.E.T.P. of less than 1 in. claimed for this design merits further study for lipid separations.[21]

Lest the reader become over optimistic concerning the potentialities of this type of equipment, however, the author has observed that conditions for optimal operation are rather critical and consist of: the solvent systems being employed, the width of the annulus between rotor and tube, concentration of feed, rate of solvent feed, position of feed, rate of settling, and speed of rotor, to name a few of the variables.

It will be demonstrated later that the number of plates required to separate a component from a binary mixture by column extraction should be much less than that calculated to be required for the fundamental procedure of countercurrent distribution. The number of extraction stages potentially available in the equipment just described should be commensurate with the difficulty of separating certain complex mixtures of lipids of industrial, or research interest.

APPLICATIONS OF COUNTERCURRENT DISTRIBUTION

The lipid chemist indeed has need of improved fractionating tools. In evidence, one can point to the rare, and exceptional instances in which pure triglycerides have been isolated from natural sources.[23] Among even the less complex free acids and monoesters, fractionation is achieved with available tools in some instances but not in others. Thus, vacuum distillation through highly developed columns, such as that of the Podbielniak design, suffices to separate the important members of the homologous series of saturated acid esters; but for the isologous series of unsaturated C_{18} acid esters, distillation procedures are not effective.[24], [25] These isologues are separable in small amounts by chromatographic methods[26], [27], [28], [29], [30] or by chemical methods on larger scale. The widely accepted bromination-debromination procedures used to isolate linoleic and linolenic acids, however, are drastic and result in formation of *cis-trans* isomers.

The present section on applications describes the use of countercurrent distribution for the fractionation of lipids primarily as an analytical tool and attempts to evaluate the efficacy of the method. Lipid compounds include the homologous series of saturated fat acids, the isologous and isomeric C_{18} acids, methyl esters of these fat acids, glycerides, fat-soluble pigments, phospholipids, bile acids, and fat acid oxidation products.

Fat acids

Countercurrent distribution of lipids has perhaps found its highest development in the work of CRAIG and his co-workers on the fractionation of fatty acids.[31]

These excellent analytical separations have been made possible by the painstaking search for selective solvent systems and by the development of the automatic countercurrent distribution apparatus described above, which is capable of applying thousands of stages.

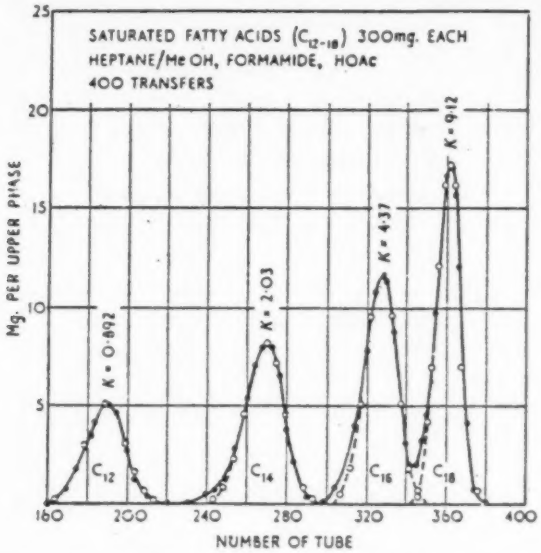


Fig. 10. Countercurrent distribution of the homologous series of higher fat acids.[31]

Separation of the higher saturated fat acids C_{12} to C_{18} is effectively complete as shown in Fig. 10 after the application of 400 transfers. The solvent system was composed of n-heptane, formamide, methyl alcohol, and acetic acid in

Table 1. Partition coefficients of fat acids and methyl esters

	Acids	*Methyl esters*
Acetic	0·09*	
Propionic	0·50*	
Butyric	2·24*	
Valeric	10·00*	
Valeric	0·061†	
Hexanoic	0·238†	
Heptanoic	1·08†	
Octanoic	4·17†	
Lauric	0·9‡	1·3§
Myristic	2·0‡	1·5§
Palmitic	4·4‡	1·9§
Stearic	8·9‡	2·3§
Oleic	4·9‡	1·8§
Linoleic	2·9‡	1·2§
Linolenic	1·6‡	0·94§

* 2·2 M phosphate buffer at *p*H 5·7, isopropyl ether.[34]
† 1 M phosphate buffer at *p*H 7·7, isopropyl ether.[33]
‡ Formamide acetic acid, methanol, heptane system.[31]
§ Nitroethane, nitromethane, pentane hexane system.[36]

volume ratios of 3 : 1 : 1 : 1, respectively. The partition coefficients for this series, as shown in Table 1, are well separated; lauric — 0·9, myristic — 2·0, palmitic — 4·4, stearic — 9·1. The R^2 values corresponding to the C_{12}—C_{14}, C_{14}—C_{16}, C_{16}—C_{18}, binary system of fat acids are 33, 106, and 384 respectively.

Countercurrent distribution renders its greatest service in the separation of the isologous series of C_{18} acids, for which satisfactory fractionating tools are generally lacking. Fig. 11 shows the virtually complete separation of linolenic, linoleic, and oleic acids following the application of 650 stages in the heptane-formamide-methyl alcohol-acetic acid solvent system. Partition coefficients calculated from this data for oleic, linoleic, and linolenic acids are 4·9, 2·9, and

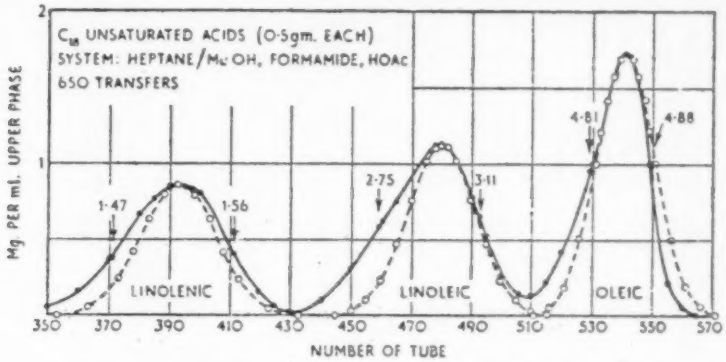


Fig. 11. Countercurrent distribution of the isologous of C_{18} acids.

1·6, respectively. R^2 values for the binary systems composed of stearic-oleic, oleic-linoleic, and linoleic-linolenic acids are 474, 328, and 118, respectively.

The automatic apparatus and the formamide, methanol-acetic acid-heptane solvent system have been applied to the analysis of the fatty acid composition of pig mesenteric fat. Not only was the spectrophotometrically determined composition of the unsaturated oleic and linoleic acids confirmed, but also myristic, palmitic, and stearic acids were isolated, and their proportions found to check with the earlier figures of HILDITCH.

In view of the number of stages required to effect the separations of unsaturated C_{18} acids, it is not surprising that DAVIES and EDWARDS, studying the fractionation of linseed fat acids, achieved only a slight fractionation in thirty stages.[32] The fractionation that was achieved leads to the belief that their 95% methanol-petroleum ether solvent system was not as selective as the heptane-formamide-methanol-acetic acid system of CRAIG.

The skewing of experimental distribution curves is a serious problem with the C_8—C_{12} fat acids in certain solvent systems, such as isopropyl ether and aqueous phosphate buffers.[33] Although distinctly useful separations were attained despite this difficulty, the mathematical analysis of countercurrent distribution does not apply rigorously. This difficulty appears to have been largely overcome by the addition of acetic acid to the solvent system (see Figs. 10 and 11). The presence of this acid is believed to lower associations between carboxyl

groups of fat acids by causing the association to occur with the acetic acid carboxyls and thus to contribute to constancy of the partition coefficient. In light of the role acetic acid appears to play in the formamide system, it should now be possible to devise solvent mixtures which will minimize skewing for the C_8—C_{12} acids.

Distribution curves for the lower aliphatic acid group, C_5 to C_8, have been determined in a system of isopropyl ether and 1 M potassium phosphate buffer at *p*H 7·7 and show insignificant skewing.[33] The partition coefficients for these acids are as follows: valeric-0·0612, hexanoic-0·238, heptanoic-1·08, and octanoic-4·17. Values of R^2 for the binary systems composed of consecutive members are: valeric-hexanoic-11·1, hexanoic-heptanoic-4·8, heptanoic-octanoic-21·2. As these values indicate, separation of the acids, C_5—C_8, is well within the capacity of limited numbers of stages such as are available in metal equipment.

Acetic, propionic, butyric, and valeric acids have also been distributed in an isopropyl ether 2·2 M phosphate buffer at *p*H 5·7.[34] The corresponding partition coefficients are 0·09, 0·50, 2·24, and 10·0. It is also apparent from Table 2

Table 2. Separability indices (R^2) for members of the homologous and isologous series of fat acids and methyl esters

Binary system	*Acids*	*Methyl esters*
Acetic–Propionic	3	
Propionic–Butyric	8	
Butyric–Valeric	75	
Valeric–Hexanoic	11	
Hexanoic–Heptanoic	5	
Heptanoic–Octanoic	21	
Lauric–Myristic	33	1,122
Myristic–Palmitic	106	647
Palmitic–Stearic	384	889
Stearic–Oleic	474	529
Oleic–Linoleic	328	169
Linoleic–Linolenic	118	293

that the separation of these acids is well within the capacity of apparatus of limited numbers of tubes.

An application of the countercurrent distribution procedure was made by ATCHLEY, who contributed evidence that fats are metabolized in the kidney through β-oxidation.[35] Propionic acid, formed in the oxidation of valeric acid by kidney enzymes, was isolated and identified by its countercurrent distribution pattern. When isocaproic acid was the substrate, isobutyric acid was identified in the oxidation products.

Methyl esters of fat acids

It is to be expected that the separation of methyl esters will be more difficult than the separation of the corresponding acids in that the addition of the common ester group increases the similarity of the molecules. No direct comparison

of the efficiency of separation, however, is possible since the same solvent system cannot be used for both acids and esters. The experimental observation that separations of methyl esters is generally more difficult than the separation of the acids is probably a reflection of a less selective solvent system available for the esters as well as of the greater inherent similarity of the ester molecules.

Replacement of the polar acid group of fat acids by the less polar ester group of fat acid esters requires the development of a solvent system in which the phase immiscible with the nonpolar hydrocarbon will itself be less polar, and thus will have increased solubility for the ester. Unfortunately, the lower the polarity of this layer, the greater will be its solubility in the nonpolar hydrocarbon layer so that a rather narrow range of solvents meets the requirements of immiscibility, favourable partition coefficients for esters, and selectivity.

An attempt to systematize the requirements of a selective solvent has been made by FREEMAN [36] According to his rule, certain ratios exist between given functional groups and the number of carbon atoms permitted in solvent molecules which will give immiscibility. Thus for aldehyde, carboxyl, and nitro functional groups, 2 carbon atoms are permitted, while for a phosphate group, 6 carbon atoms are permitted in the remainder of the molecule.

A solvent system developed for glyceride separations is also useful for methyl monoesters and illustrates the more or less empirical manner in which solvent systems are developed.[37] Nitromethane is immiscible with pentane-hexane at room temperature but possesses low differential solubility for methyl esters. Nitroethane has much improved differential solubility characteristics at 0°C, but is miscible with pentane-hexane at room temperature. A mixture 80% nitroethane and 20% nitromethane was found to be immiscible with pentane-hexane at room temperature and at the same time to yield partition coefficients close to 1 for both glycerides and monoesters.

Countercurrent distribution curves for isologous series of C_{18} acid methyl esters in this solvent are given in Fig. 12 and for the homologous series of saturated acid methyl esters in Fig. 13. In Table 1 are given the partition coefficients of fat acids and methyl esters; in Table 2 are given the R^2 values calculated for certain binary systems of interest. It is apparent that separation as free acids is, with one exception, more attractive than as methyl esters; however, when separations must be performed upon esters, the nitroparaffin system can be resorted to.

The number of extraction stages required as calculated above (R^2) is for the fundamental countercurrent distribution operation. It should be pointed out that this calculation has little relationship to the number of stages required for continuous countercurrent extraction to separate a binary system into raffinate and extract fractions. This point may be illustrated by the data shown in Fig. 2. Although 53 plates were applied to give an 84% separation of chlorophyll *a* and *b*, the effective fractionation was taking place in 13 extraction stages or tubes 17–29, inclusive. Beyond these tubes the separations are effectively complete, and single components are present.

A truer contrast between the fundamental operation and continuous multiple-stage countercurrent extraction with continuous feed is given in Fig. 14. In this instance, solvent volumes in the tubes were adjusted to give symmetry of

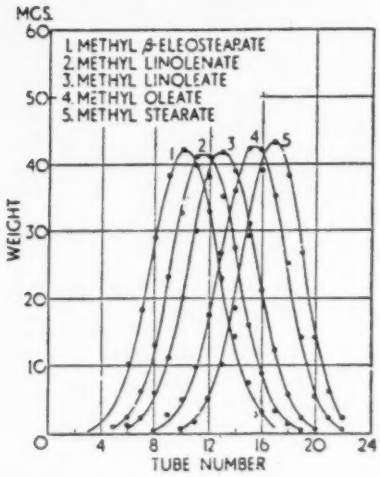


Fig. 12. Countercurrent distribution of the C_{18} acid methyl esters. (1) Methyl β-eleostearate. (2) Methyl linolenate. (3) Methyl linoleate. (4) Methyl oleate. (5) Methyl stearate.

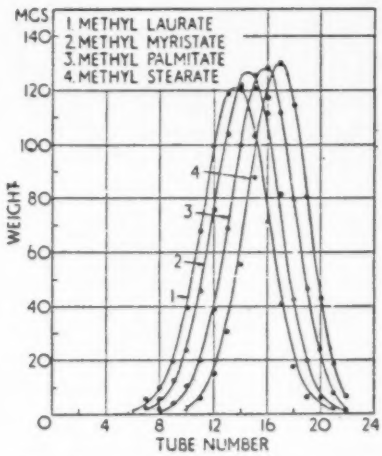


Fig. 13. Countercurrent distribution of the homologous series of higher fat acid methyl esters. (1) Methyl laurate. (2) Methyl myristate. (3) Methyl palmitate. (4) Methyl stearate.

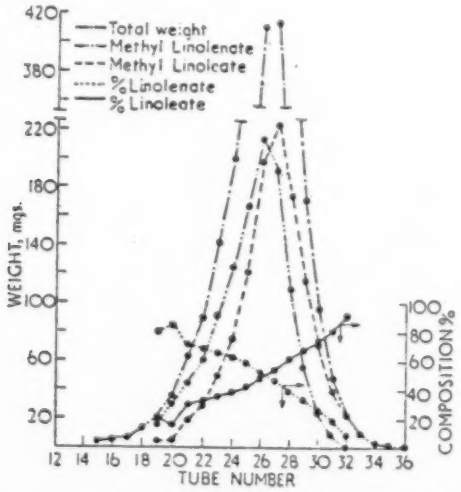


Fig. 14. Countercurrent extraction with continuous feed of a methyl linoleate and methyl linolenate mixture.

separation about the point of feed. The adjusted "partition coefficients" for linoleate and linolenate were 1·11 and 0·9 and thus reciprocals. An approximately equal mixture of linoleate and linolenate was introduced into the Craig apparatus after every second transfer at a tube midway between upper tube O

and the starting position. Fifty-three transfers and 26 introductions of esters were made in order to approximate column operation at equilibrium conditions. It is apparent that separation of the esters is 99% or more complete in tubes which are removed 5 or more stages either side of the centre point of feed. The 10 to 11 tubes required in this type of operation is far less than the 234 transfers calculated for the fundamental operation in which high purity samples at either end of the distribution are united with 50% purity samples in the middle of the distribution for the purposes of statistical calculations.

While this important consideration might appropriately have been brought out under the section describing continuous countercurrent extractors, it has been discussed here, in part because the Craig countercurrent distribution apparatus was used for the experiments and in part because of the desirability of an early clarification of the relationships between the fundamental operation of countercurrent distribution and the continuous countercurrent extractions of commercial interest.

Glycerides

Interest in the fractionation of glycerides stems in part from the need to intro-convert fats and make a given fat a source of glycerides for specific uses. Thus in periods of tung oil shortage, a substitute oil may be found in fractionated linseed oil. The suitability of soybean oil both for a paint oil and for edible oil purposes is enhanced by fractionation into a low iodine and a high iodine value component. Under this concept of interchangeability, fats are looked upon merely as a source of fat acids to be fractionated and recombined as the specific use requires.

A single-stage, multiple extraction of soybean oil to obtain a paint oil has been reported by *Edeleanu*, who used liquid sulphur dioxide as solvent.[39] FREEMAN has evaluated the selectivity of 28 solvents by the single-batch extractions of soybean oil.[36] He determined the portion of oil extracted by the immiscible solvent and iodine value difference between the portions. KLEINSCHMIDT and KRAYBILL report the single-stage multiple extraction of soybean oil to give fractions ranging in iodine value from 140 to 108.[40]

Only one paper employing countercurrent distribution for the fractionation of glycerides has been reported.[37] A 24-plate distribution of soybean oil between pentane-hexane and furfural is presented in Fig. 15. Iodine values of the fractions ranged between 107 and 163. The data demonstrates that this method has potentialities for the analytical separation of naturally occurring glycerides, particularly if increased numbers of transfers available in CRAIG's automatic equipment are applied.

The separability of glycerides is limited by their inherent structure. As shown in Table 3, the linolenic acid-free glycerides of soybean oil could theoretically be isolated in a low iodine value fraction (I.V. 120) comprising 72·1% of the original oil if the fat acids of soybean oil were distributed according to the "even" pattern.[3] However, according to the "random" pattern,[42] only 7·9% of the glycerides with an iodine value of 49 could be isolated free of linolenic acid.

Table 3. Theoretical separations permitted under monoacid, even, and random distribution patterns

Distribution pattern	Linolenic acid free fraction		Linolenic acid containing fraction	
	% Yield	Iodine value	% Yield	Iodine value
Monoacid . . .	91·0	122·5	9·0	261·6
Even	72·1	120·0	27·9	175·0
Random . . .	7·9	49·0	92·1	142·4

Countercurrent extraction studies have contributed to our knowledge of the glyceride structure of soybean oil. The results are best presented in the form of Fig. 16 where iodine value is plotted against per cent yield of the lower iodine value component (raffinate) on the bottom abscissa, and against per cent yield

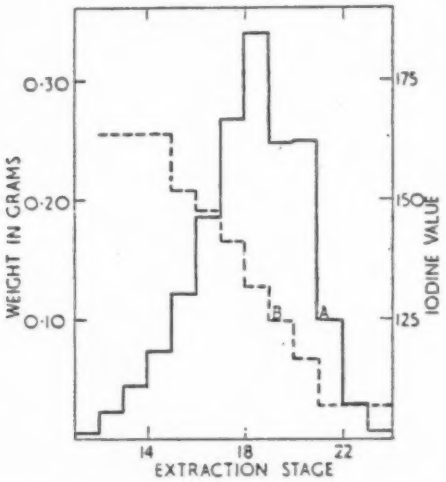


Fig. 15. Countercurrent distribution of soybean glycerides between hexane and furfural. (*A*) Weight. (*B*) Iodine value.

of the higher iodine value component (extract) on the top abscissa. The separations theoretically obtainable for raffinate and extract components are represented by curves *A* for the random pattern and by curves *B* for the even pattern. The curves indicate that for 50% yield of the high and the low iodine value fraction, iodine values of approximately 166 and 90 are possible under both even and random patterns. Failure of experimental data to approach these limits may most probably be interpreted as a measure of the inefficiency of the fractionating tools. The crossing of the curve for the nitroparaffin-hexane countercurrent extractions over the limit (lower curve *B*) imposed by the even distribution theory indicates that soybean glycerides are not arranged according to a strictly even pattern. It may be noted that the experimental curves do not closely approach the limiting curve imposed by the random distribution pattern. This may be due either to insufficient fractionating power or to a pattern of

distribution other than random; e.g. partial random.[43], [44] The conclusion that soybean oil is not distributed in a strictly even pattern is of vital importance to the commercial processes for the fractionation of soybean oil into paint and edible oil fractions. Data for pilot-plant separations are also represented in

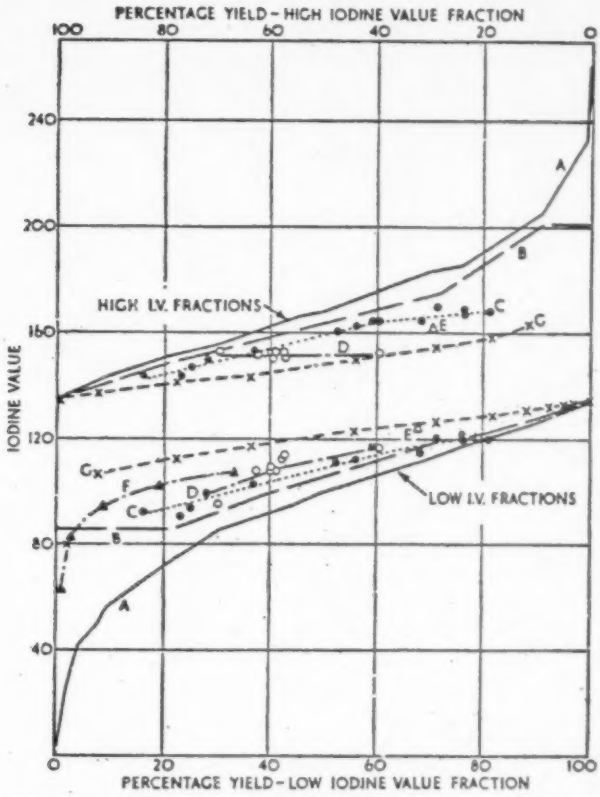


Fig. 16. Experimental fractionations by countercurrent extraction and theoretical separations of soybean glycerides. (*A*) Random pattern. (*B*) Even pattern. (*C*) N.R.R.L. pilot plant. (*D*) P.F.G. pilot and commercial plants. (*E*) "Solexol" pilot plant. (*F*) Laboratory nitroparaffin-petroleum ether countercurrent distribution. (*G*) Laboratory furfural-hexane countercurrent distribution.

Fig. 16 and will be discussed under the multiple contact, continuous countercurrent extraction processes section.

Phospholipids

New information on the composition of soybean phosphatides has resulted from the application of countercurrent distribution procedures and a hexane-90% methanol solvent system. Comparatively recent literature has given the composition of "soybean lecithin" as 35% lecithin and 65% cephalin[45], [46] even after the discovery of soybean phosphoinositides.[47] As a result of countercurrent distribution studies, it has been found that the so-called "cephalin" is

largely phosphoinositide and that cephalin, phosphatidyl ethanolamine, contrary to previous work is to be found in the alcohol-soluble fraction.[48] It can now be estimated that soybean phosphatides are composed of 29% lecithin, 31% cephalin, and 40% phosphoinositides.

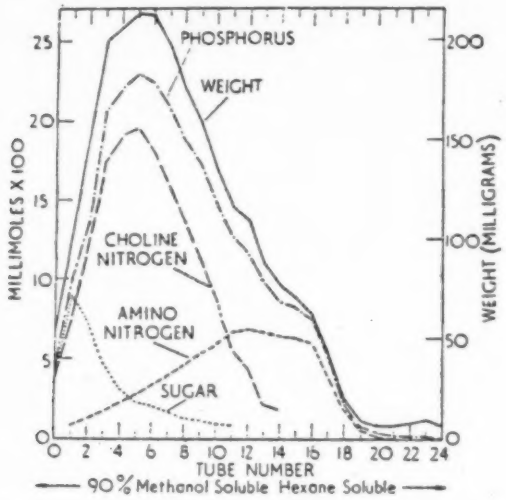


Fig. 17. Countercurrent distribution of the alcohol-soluble fraction.

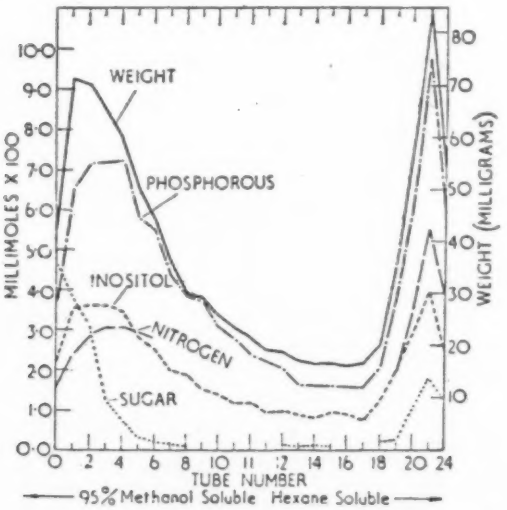


Fig. 18. Countercurrent distribution of the alcohol-insoluble fraction.

The countercurrent distribution patterns for the alcohol-soluble and the alcohol-insoluble phosphatides are given in Figs. 17 and 18. In this instance, it may be seen that the curves deviate greatly from the theoretical form. Association between the various phosphatides undoubtedly takes place and distorts the

shape of the curves. Despite this deficiency, useful separations are obtained which have permitted estimation of composition, revealed new phosphoinositides, and suggested new fractionating methods for the study of the phosphoinositides.

Sugars are to be found in the countercurrent patterns for both the alcohol-soluble and the alcohol-insoluble fractions. This sugar exists in a free, and in a chemically combined form. The free sugar of the alcohol-soluble fraction is primarily sucrose (87%) with a small amount of raffinose (9%), while that of the alcohol-insoluble is primarily stachyose (79%) with a small percentage of raffinose (12%). Free sugars may be removed from the phosphatides by extract-

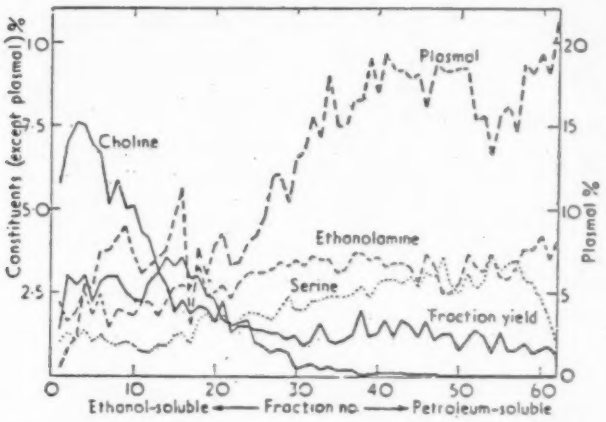


Fig. 19. Distribution of crude ox brain glycerophosphatides.[52]

ing petroleum ether solutions with 50% ethanol. Combined sugars associated with the hexane-soluble phosphoinositides are freed only after hydrolysis with acid. They were found to consist of arabinose, galactose, and mannose.[49]

Comparison of the countercurrent distribution patterns for soybean phosphatides with those of linseed[50] and corn[51] reveal a qualitative similarity of the phosphatides from these diverse sources. All possess lecithin, cephalin, and two phosphoinositides. The hexane-soluble phosphoinositide fraction of corn has the same three combined sugars as that of soybeans: namely, arabinose, galactose, and mannose.

In a recent publication, LOVERN has applied the countercurrent distribution procedure to ox brain phospholipids.[52] Twenty conical flasks were chosen rather than separatory funnels or the Craig apparatus. The 85% ethanol fractions were collected after successive contacts with petroleum ether layers contained in the flasks. A useful separation of phosphatidyl choline from similar lipids containing ethanolamine and serine was obtained; however, the separation of phosphatidyl ethanolamine from phosphatidyl serine was poor (Fig. 19). Several lipids not so far classifiable were found.

COLE, LATHE, and RUTHVEN have also reported a countercurrent fractionation of lipid material from brain. They used a solvent system composed of carbon

tetrachloride 62%, methanol 35%, and water 3·15%.[53] Sphingomyelin was found in fraction 21; lecithin, in 27; cerebroside, in 29; cholesterol, in 35; and neutral fat and non-polar material, in 45.

While these studies have added to knowledge of phosphatide composition, it is apparent that new solvent systems must be devised in which association is

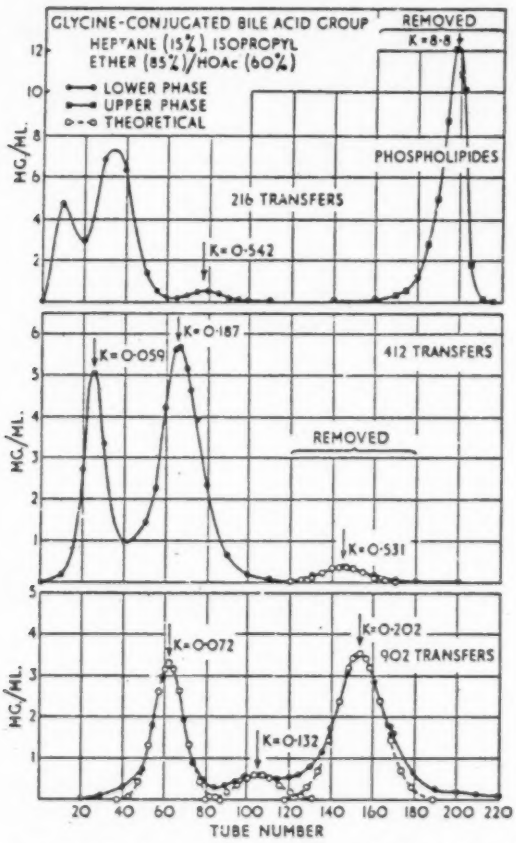


Fig. 20. Distribution pattern of glycine-conjugated bile acids.

minimized, in order to segregate components more perfectly. Moreover, solvent systems must be devised in which partition coefficients may be adjusted closer to 1 for the specific major components of interest. A suggestion for minimizing association is apparent in the work of CRAIG on bile acids, discussed below. In that solvent system, phospholipids gave a normal distribution curve (Fig. 20).

Bile acids

Problems in the study of bile acids by countercurrent distribution were much like those encountered in the study of the phospholipids. In the first place, some information as to the identity and structure of major components was known, but until the countercurrent distribution work of AHRENS and CRAIG,[54] there had

been no "all-inclusive analysis" which this technique provides. In further similarity to the phosphatide analysis problem, preliminary fractionations of the crude biological mixture were found advisable prior to submitting the samples to intensive countercurrent distribution. Bile acids like the phosphatides present an exaggerated case of association of components. Based on the discovery, during the fatty acid distribution work, of methods by which associations may be minimized, AHRENS and CRAIG incorporated acetic acid into the solvent mixture. Carboxyl groups of the bile acids are thus associated with acetic acid carboxyls rather than with themselves. Normal distribution curves for many of the bile components were thereupon achieved as shown in Fig. 20. The analysis of the glycine-conjugated bile acid group given in the upper section of the figure demonstrates the presence of phospholipids which were removed from the apparatus in accordance with the single withdrawal technique in the course of applying 216 transfers. Phosphatides also give a normal curve in this solvent system. Application of a total of 412 transfers resulted in the removal of a bile acid of partition coefficient 0·531 as indicated in the middle section of the graph. Finally with the application of 902 transfers, the distribution of the lower section of the graph was obtained which reveals the presence of three components. Those of partition coefficients 0·202 and 0·072 have been tentatively identified as glycodesoxycholic acid and glycocholic acids; the other two constitutes are presumably new compounds. The taurine-conjugated bile acids (not shown here) gave a distribution pattern of greater complexity. Taurocholate and taurodesoxycholic acid were identified on the basis of their partition coefficients.

Pigments

Chloroplast pigments have long been isolated and characterized by virtue of differences in their partition coefficients. In early isolation procedures for chlorophyll *a* and *b*, WILLSTÄTTER and STOLL relied upon the greater solubility of the *a* component in a petroleum ether layer at equilibrium with 85% methanol and the greater solubility of chlorophyll *b* in the alcoholic layer.[55] After extracting chlorophyll *b* from a petroleum ether layer with successive batches of alcohol and back extracting the alcoholic extracts with petroleum ether to a limited extent, the alcoholic extracts were worked up for chlorophyll *b* and petroleum ether hyperphases for chlorophyll *a*.

The differential solubility characteristics of carotenoid pigments have in a similar manner served both for the isolation of pigments and also for their characterization and differentiation.[56] In recent years, partition coefficients have been published for highly purified carotenoid pigments and have served as a basis for specifying analytical procedures.[57]

One paper has appeared describing the systematic countercurrent distribution of plant pigments.[6] In this instance, the distribution of chlorophyll *a*, chlorophyll *b*, and carotene was studied. These pigments comprised a model system upon which certain applications of statistical theory described above were tested. They present a graphic visual demonstration of the countercurrent distribution process as in the course of the operation, the yellow carotene

pigment segregates from the blue-green chlorophylls, and later as the chlorophyll tubes differentiate into the yellow-green chlorophyll *b* and the blue chlorophyll *a* components. Data for a 24-plate distribution are represented in Fig. 21 and indicate the potentialities of liquid-liquid extraction for isolation and characterization purposes when carried out in a systematic procedure.

Chromatographic methods of pigment isolation have all but replaced the earlier solvent distribution methods. The high resolution of the chromatographic column, equivalent to hundreds of extraction stages, overshadows the disadvantages present in chromatography in its lack of reproducibility, its irreversible adsorption, and nonlinear adsorption isotherms. However, with the

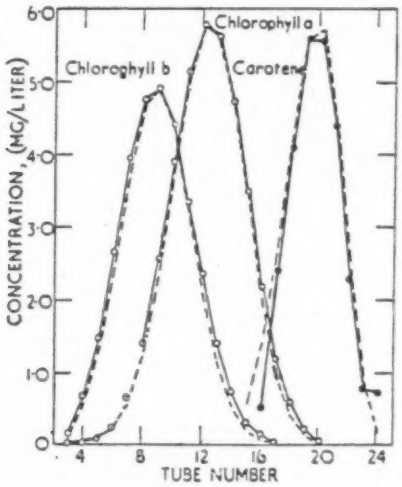


Fig. 21. Countercurrent distribution of chlorophyll *a*, chlorophyll *b*, and carotene in the 25-tube Craig distribution apparatus. Solid lines represent experimental data; broken lines show the calculated theoretical distribution.

development of efficient laboratory and commercial scale countercurrent extractors, the merits of partition procedures will need to be reconsidered. Solvent partition procedures may yet have a valid position in meeting the recent demands for chlorophyll, carotene, and other plant pigments.

Fat acid oxidation products

Countercurrent distribution techniques are particularly attractive for the fractionation of labile fat acid oxidation products. Subtle changes such as *cis-trans* isomerization of double bonds and the complex interactions of unstable oxygen adducts are all minimized by the low temperature, dilute solution and otherwise mild conditions of solvent partition procedures. Sufficient studies in this field have been published to demonstrate the suitability and applicability of the technique, but the most significant contributions undoubtedly lie in the future.

In the course of studying interferences in the spectrophotometric method for fat acid composition, PRIVETT and LUNDBERG[98] found that autoxidized methyl

esters of fat acids could be separated from the unoxidized esters by a modified countercurrent extraction procedure using separatory funnels. Skellysolve F and 87% ethanol were used as solvents. In a later publication, PRIVETT, LUNDBERG, and NICKELL described the quantitative separation of the oxidized from the unoxidized fractions of autoxidized methyl linoleate, linolenate, and oleate.[59] The structure of hydroperoxides obtained from autoxidized methyl linoleate by this modified countercurrent extraction procedure has been studied recently by PRIVETT, LUNDBERG, KHAN, TOLBERG, and WHEELER.[60] These results are reviewed in chapters on infrared spectroscopy and autoxidation.

The countercurrent distribution behaviour of various model compounds of interest to fat acid oxidation has been described for an 80% ethanol-hexane system.[61] For example, the effect upon the partition coefficient of methyl stearate of introducing an epoxide, a keto, a hydroperoxide, and one and two hydroxy functional groups is shown in Table 4 along with other compounds of

Table 4. Partition coefficients and positions of maxima for various model compounds

Compound	*Partition coefficient*	*Tube number**	*Functional groups*
Azelaic acid	0·05	1	di—COOH
Dihydroxy methyl stearate	0·17–0·21	3, 4	di—OH and COOR
12-Hydroxy stearic acid	0·20	4	OH and COOH
Monoglyceride of cottonseed oil	0·28	5	di—OH
Methyl oleate hydroperoxide	0·57	9	OOH and COOR
Hydroxy methyl stearate	0·97–1·01	11, 12	OH and COOR
Methyl ricinoleate	1·01	12	OH, =, COOR
Heptenal	2·05	16	= and C=O
Keto methyl stearate	2·22	17	$-R_2C=O$ and COOR
Nonenal	2·96	18	= and C=O
Stearic acid	3·36	18	COOH
Epoxy methyl stearate	4·40	20	C—C and COOR (O)
Methyl oleate	5·15	20	= and COOR
Diglyceride of cottonseed oil	10·76	22	OH
Methyl stearate	19·75–19·31	23	COOR

* Distribution (24-transfer) between hexane and 80% ethyl alcohol.

interest. The more polar the functional group the greater the lowering of the partition coefficient; epoxy, keto, hydroxy, hydroperoxide, and two hydroxyl groups give increased lowering in the order listed. Compounds studied behaved almost ideally in the concentrations employed.

The autoxidation process in methyl oleate[62] as revealed by countercurrent distribution studies is quite comparable to that of methyl linoleate, and for that reason, only the studies on methyl linoleate[63] need to be discussed here. Investigations on methyl linoleate also have the added advantage that this ester shows ultraviolet and infrared absorption phenomena not possessed by methyl oleate.

In agreement with previous studies on the autoxidation of methyl linoleate,

1 mol of hydroperoxide was found to be formed for each mole of oxygen absorbed at low oxidation levels; e.g. 0·3 mol oxygen per mole ester and below. By countercurrent distribution methods, it was possible, moreover, to actually isolate 1 mol of hydroperoxide for each mole. of oxygen absorbed. The hydroperoxides thus isolated were obtained in Ca., 93% purity as measured by ultraviolet absorption and peroxide value determinations. The infrared absorption curves of such a methyl linoleate hydroperoxide preparation is shown at (c) in Fig. 22. The absorption peak at 2·9μ is that due to the hydroperoxide group. Of particular interest are the twin valleys at 10·12 and 10·52μ which structure is characteristic of *cis-trans* conjugated dienes.[64] This observation has particular significance to the mechanism of autoxidation. Not only does it confirm the observation that oxygen absorption shifts double bonds to conjugated positions and causes the bond shifted to change from *cis* to *trans* configuration,[65] but, equally important, it suggests that the ultraviolet absorption coefficients selected be those of *cis-trans* conjugated dienes rather than those of *trans-trans* conjugated dienes as commonly employed. (See also the chapters on infrared spectroscopy and autoxidation.)

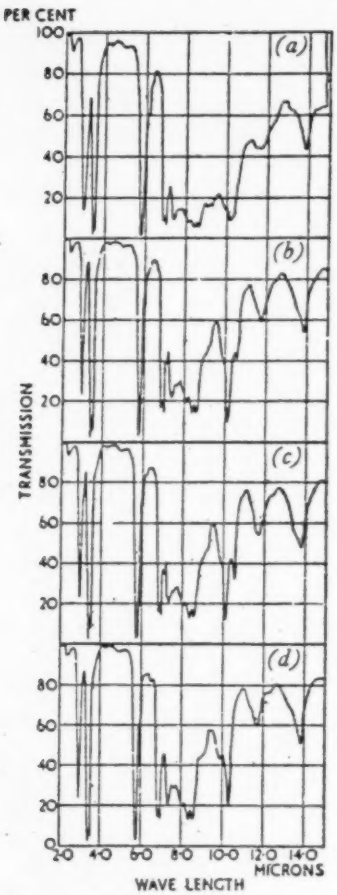


Fig. 22. Infrared absorption spectra of (*a*) the secondary oxidation product (Component III) of methyl linoleate, tube 2-room temperature autoxidation; (*b*) methyl linoleate hydroperoxide, tube 9-room temperature autoxidation; (*c*) methyl linoleate hydroperoxide, tubes 5–14 inclusive 0°C autoxidation; (*d*) Methyl oleate hydroperoxide refractionated by countercurrent distribution.

When a coefficient appropriate for *cis-trans* conjugated dienes is applied to ultraviolet absorption data, a striking conclusion is indicated. Instead of being two-thirds conjugated in accordance with the accepted free radical theory of the mechanism,[66] methyl linoleate hydroperoxide is more than 90% conjugated. If nonconjugated hydroperoxides were present, they would be indicated by a peak at 10·3μ characteristic of the isolated *trans* bond; e.g. as at 22d for methyl oleate hydroperoxide (more correctly perhaps methyl elaidate hydroperoxide.[65]*) The absence of nonconjugated hydroperoxides now indicated is in agreement with the observation of BERGSTRÖM that 9 and 13

* It is of interest to note that while countercurrent distribution of a sample of methly oleate hydroperoxide provided by Dr. D. SWERN did not greatly raise the iodometric peroxide value, it did sharpen the infrared absorption bands and removed background absorption.

hydroxystearate but not 11 hydroxystearate could be isolated after the reduction of linoleate hydroperoxide.[107]

In Fig. 23 is given the countercurrent distribution pattern for methyl linoleate oxidized to the 0·62 mol oxygen per mole ester level. The material in the peak to the right is unoxidized methyl linoleate; the central peak is that for methyl linoleate hydroperoxide, and the third peak is that of secondary oxidation products.

Methyl linoleate isolated from the fractions to the right shows all the characteristics of the original unaltered ester, including partition coefficient, hydrogen absorption, hydroxyl value, iodine value, and infrared absorption. The hydroperoxide fraction possesses high levels of diene conjugation, one peroxide group

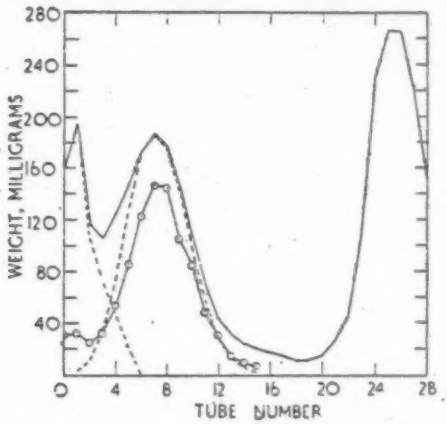
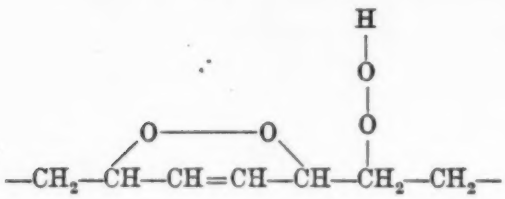


Fig. 23. Weight-distribution curve (solid line) following countercurrent fractionation of autoxidized methyl linoleate (0·62 moles oxygen absorbed per mole ester—light catalyzed). Circles indicate weight of diene-conjugated esters. The broken line is the theoretical curve, while the dashed line is the difference curve.

per mole, which forms one hydroxyl group on reduction; it absorbs 3 mol of hydrogen (2 mol for the double bonds and one for the hydroperoxide) and has the molecular weight anticipated for monomeric peroxides.

The third peak for secondary oxidation products is nearly absent in the countercurrent distribution patterns of linoleate oxidized to low levels (0·3 mol and less). It is possible to calculate the moles oxygen in this component since there is virtually no loss of material on countercurrent distribution and since the moles oxygen absorbed by the mixture and the moles present in the hydroperoxide component are known. With these considerations, it is calculated that this fraction possesses 2 mol or more of oxygen per mole ester, dependent on the level of oxidation. This secondary oxidation product mixture is primarily monomeric but has an appreciable acid content. Experimental facts are consistent with the following formula for the major component of this fraction, if the ring structure is stable under conditions of peroxide determination and hydrogenation.



The experimentally observed loss in diene conjugation (both ultraviolet and infrared), the single mole of peroxide found, the 2 mol of hydrogen absorbed to

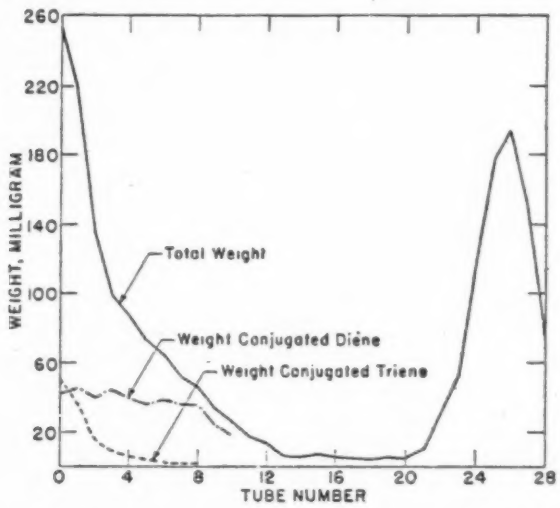


Fig. 24. Weight distribution curve following countercurrent fractionation of autoxidized methyl linolenate. Calculated diene and triene quantities expressed as weights of methyl linolenate converted to conjugated form.

yield one hydroxy group, and the monomeric molecular weight is thus accounted for.

The autoxidation process for methyl linolenate as revealed by countercurrent distribution stands in sharp contrast to that for methyl oleate and methyl linoleate.[68] For example, no peak was found corresponding to a monomeric hydroperoxide of methyl linolenate. Oxygen addition is immediately followed by polymerization to give first an unstable diene conjugated dimer; then follow polar diene- and triene-conjugated polymers. Less than half of the linolenate actually oxidized is converted to conjugated form, while more than half of the double bonds originally present are destroyed, probably through polymerization initiated by oxidative attack on the ethylenic bonds. A partial fractionation of triene-containing polymers from diene-containing polymers can be made by countercurrent distribution as shown on the left in Fig. 24, and a distinct separation of unoxidized methyl linolenate on the right.

The procedure of subjecting autoxidized esters of fat acids to countercurrent distribution and of subsequently measuring the infrared absorption spectrum of

fractions has been followed by LEMON, KIRBY, and KNAPP[69] in the case of peanut methyl esters. Differences in absorption spectrum, that were observed to exist between fractions, and between fractions and the autoxidation mixture, are provocative of further research.

An interesting and useful separation of autoxidation products from undecylenic acid is reported by DAL NOGARE and BRICKER.[70] After saponification and acidification of an autoxidation fraction, undecylenic acid was recovered in a petroleum ether soluble fraction. The insoluble fraction was distributed in 9 separatory funnels between 40% methanol and ethyl ether to give the separation shown in Fig. 25. Pure 10–11 dihydroxy-hendecanoic acid was isolated from

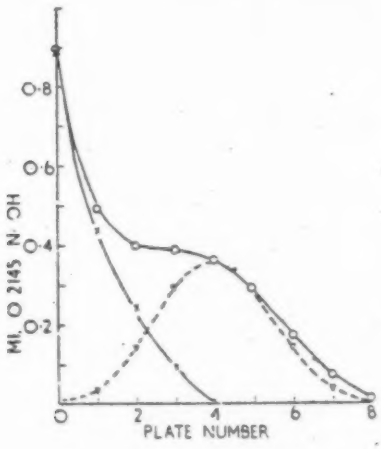


Fig. 25. Distribution curve for fraction containing 10, 11-dihydroxyhendecanoic acid. Circles indicate experimental data; triangles, the theoretical curve for 10, 11-dihydroxy-hendecanoic acid; and crosses, the calculated curve for impurity.[70]

funnels 4–8. The impurity of tubes 0–4 appeared to be an aldol type condensation product from which sebacic and azelaic acids could be isolated following permanganate oxidation.

Countercurrent distribution data frequently suggest methods for large scale preparations. These larger scale operations may be continuous columnar operations or multiple-batch operations such as the operation to be discussed next.

One example of the application of solvent distribution data to larger scale operations is that of the preparation of methyl linoleate hydroperoxide.[71] Methyl esters of safflower oil in 2 kg batches oxidized to a level of 0·1 mol peroxide per mole ester were distributed between pentane-hexane and 80% ethanol. Three 5-gal bottles were used, each of which contained 10 litres of pentane-hexane and the first of which contained in addition the oxidized esters. Four 10-litre batches of 80% ethanol were successively equilibrated with the pentane-hexane layers in the bottles, mixing of phases being accomplished by air-driven stirrers and transfer of the lower phase being accomplished by

siphoning. After collection of the aqueous-ethanol extracts, hydroperoxides were isolated by diluting the alcohol concentration to 50%. A shallow pentane-hexane solution of the hydroperoxides thereupon separated and was removed. Using this procedure, three batches of esters were handled in a period of 6 days to prepare 700 g of hydroperoxide. This amount proved more than adequate for its testing as a catalyst in Redox recipes for the cold polymerization of rubber. It was found to be the equivalent of currently used catalysts on a molar basis.[72]

Applications of Multiple Contact, Continuous Countercurrent Extraction

Discussions of commercial operations are necessarily incomplete in that much of the information known about the processes is not available through scientific publications, but resides in the files of industrial research groups. Fortunately, several of the successful extraction processes have been described, and the potentialities of other operations less well known can be partially assessed from these.

Perhaps the best known process of liquid-liquid extraction in the lipid field is that in operation at the *Pittsburgh Plate Glass Company*.[73] Vegetable oils are extracted with furfural in vertical packed columns. Furfural is fed in at the top of the column; the oil to be fractionated, at the middle; and the reflux feed which may, or may not contain naphtha is introduced at the bottom. The low iodine value oil layer (raffinate) containing naphtha is removed at the top of the column; the furfural extract containing the high iodine value oil (extract) is removed at the bottom. In accordance with the reflux principle of distillation, a portion of this extract is returned to the bottom of the column to improve the efficiency of the separation.

This process has been in commercial operation for several years handling as much as a tank car of oil per day. Soybean oil has been fractionated to produce an extract oil for paint purposes and a raffinate oil for edible purposes; linseed oil has been fractionated to produce a substitute perilla oil and a number of speciality items.

Many factors affect the efficiency of fractionation of this process. Increasing column height, solvent and reflux ratios, and decreasing column diameter improve the efficiency. Some comparative idea of the efficiency of these operations is given in Fig. 16, where data from Gloyer *et al.* is plotted with data obtained in the pilot plant of the Northern Regional Research Laboratory.

Also plotted on this graph are one pair of points for the "Solexol Process."[74] While this process finds greatest application in the decolorization of tallows, it does have interest for glyceride fractionation. In this process oils are extracted countercurrently with liquid propane under pressure. A temperature gradient is maintained from top to bottom of the column. Glycerides of greater unsaturation tend to be less soluble in liquid propane than the more saturated glycerides. Thus the low iodine value raffinate comes off the top and the more unsaturated extract from the bottom.

This process has found greatest applicability in the removal of trace substances

from oils which differ greatly in molecular weight from the triglycerides. Solvent refining to remove colour bodies, free fatty acids, phosphatides and sterols is one of the interesting applications. A propane extraction plant has been established in South Africa for the recovery of vitamin A from fish oils.

A future development appears to reside in application of the Podbielniak type centrifugal extractor to lipid fractionation problems. This equipment is finding application to petroleum oil refining and is established in the field of recovery of antibiotics from growth media. It is currently employed in the lipid field only for the recovery of vitamins A and D from fish oils.[75] However, there would appear to be no reason why this equipment should not perform admirably the liquid-liquid extractions now being performed by columnar equipment.

In general, it may be observed that only a few commercial applications of liquid-liquid extraction to lipids can be considered at this date as established; it is apparent, however, that potentialities are presenting themselves, and many are on the verge of becoming realities. It may be expected that, as fundamental studies of countercurrent distribution point the way, we shall come to think of the various vegetable and animal fats and oils not for their individual peculiar characteristics which recommend them for specific uses, but merely as a source of the component acids with which to synthesize a fat of specified characteristics.

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22A